Blood pressure variability: measurement and clinical implications

by

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BExSc, MA, MRes

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Declarations by Author

Originality

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution, except by way of background information and of which is duly acknowledged in the thesis. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright. I have also acknowledged, where appropriate, the specific contributions made by co-authors of published and submitted manuscripts.

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Ethical Conduct

All research associated with this thesis abides by the international and Australian codes on human and animal experimentation, and full ethical approval from the relevant institutions was obtained for all studies outlined in this thesis. All individual participants provided written informed consent for involvement in the respective research studies.

Panagiota Veloudi

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Contributions to Papers Contained Within This Thesis, Publications by the Author and Statement of Co-author

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The following papers are incorporated into chapters of this thesis and were either published or submitted for publication in peer reviewed scientific journals during the course of candidature:

Chapter 1

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Author contributions:

Veloudi P.	Study conception and design, literature review, analysis and interpretation, manuscript preparation
Sharman JE.	Study conception and design, critical manuscript revision

Chapter 2

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Veloudi P., Blizzard L., Head A.G., Abhayaratna WP., Stowasser M., Sharman JE. Blood Pressure Variability and Prediction of Target Organ Damage in Patients With Uncomplicated Hypertension. *Am J Hypertens*. 2016;29(9):1046-54.

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Chapter 4

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Chapter 5

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Chapter 6

Veloudi P., Blizzard L., Ding CH., Cicuttini FM., Jin X., Wluka AE., Winzenberg T., Jones G., Sharman JE. Effect of Vitamin D Supplementation on Aortic Stiffness and Arterial Hemodynamics in People With Osteoarthritis and Vitamin D Deficiency. *J Am Coll Cardiol.* 2015 15;66(23):2679-81.

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Appendix 4

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Abstracts and Presentations at Scientific Conferences That Relate To This Thesis

The following abstracts relate specifically to this thesis and were presented at national and/or international scientific conferences during the period of candidature.

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Veloudi P., Blizzard L, Srikanth V., Sharman JE. In-clinic blood pressure reactivity is associated with preserved endothelial function among patients with diabetes. Athens, Greece (2014) International Society of Hypertension and European Society of Hypertension Combined Scientific Meeting. **Oral.**

Veloudi P., Blizzard L., Abhayaratna WP., Marwick TH., Sharman JE. Blood pressure variability and target organ damage in patients with uncomplicated hypertension: average 24 hour ambulatory BP is more relevant to changes in left ventricular mass index. Maastricht, The Netherlands (2014) Association for Research into Arterial Structure and Physiology. **Poster.**

Veloudi P., Ding CH1, Blizzard CL1. Jones G., Cicuttini FM., Wluka AE., Winzenberg T., Sharman JE. Effect of vitamin D supplementation on aortic stiffness and central haemodynamics in older individuals with vitamin D deficiency: promising observational data are not supported when tested by double-blind, placebo-controlled, randomised trial design. Maastricht, The Netherlands (2014) Association for Research into Arterial Structure and Physiology. **Oral.**

Veloudi P., Blizzard L., Srikanth V., Schultz M., Sharman JE. The assumption that blood pressure decreased over consecutive measurements is false: major implications for hypertension diagnosis and guidelines. Krakow, Poland (2014) Association for Research into Arterial Structure and Physiology. **Poster.**

Veloudi P., Ding CH1, Blizzard CL1. Jones G., Cicuttini FM., Wluka AE., Winzenberg T., Sharman JE. Effect of vitamin D supplementation o peripheral and central BP, visit-to-visit BPV and arterial stiffness in older individuals with vitamin D deficiency. Adelaide, Australia

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Veloudi P., Blizzard L., Velandai KS., Schultz MG., Sharman JE. Influence of blood pressure level and age on within-visit blood pressure variability in children and adolescents: Results from the 2011-2013 Australian Health Survey. Seoul, Korea (2016) International Society of Hypertension Scientific Meeting. **Asian Pacific Society of Hypertension Young Investigator Award. Oral.**

Veloudi P., Blizzard L., Velandai KS., Schultz MG., Sharman JE. Prevalence of pre-hypertension in children and adolescents using simplified versus complex assessment criteria. Seoul, Korea (2016) International Society of Hypertension Scientific Meeting. **Poster.**

Abstract

High blood pressure (BP), measured conventionally using clinic, ambulatory or home BP monitoring, is associated with target organ damage; however, the exact underlying mechanisms are not clear. A hypothesis which has gained wide acceptance during the last decade supports the notion that variability in BP (BPV) could provide important clinical information, over and above mean BP levels, and therefore could be relevant to the diagnosis and management of hypertension. Key gaps in the literature relating to BPV are that the prognostic significance of BPV as well as its impact on hypertension diagnosis and effect of treatment are unclear. The overall aims of this project were to investigate the effect of BPV on target organ damage in specific populations, to determine the impact of BPV on hypertension diagnosis, and to determine the effect of a novel intervention on BPV.

Data supporting the notion that BPV may offer independent prognostic value are inconsistent, and this may be due to the wide variety of methodologies used for measuring BPV. BPV can be quantified from short-term BP monitoring (using 24-hour BP), mid-term BP (using home BP in the morning, evening or day-to-day) or long-term BP monitoring (using visit-to-visit clinic BP). Study 1 (chapter 1), aimed to examine the effect of BPV methodologies on the magnitude of BPV itself, as well as the effect of participant characteristics on BPV. Key methodological factors assessed were 1) the number of BP readings or visits used to quantify BPV and 2) the duration of BP monitoring. Following a scoping review process, data were extracted from 102 studies. The novel findings of this study were that the methodology used to quantify BPV, as well as age and mean BP level, affects the magnitude of BPV itself. This underscores the need to standardize BPV protocols, particularly regarding the number of BP readings and visits.

Study 2 (chapter 2), aimed to determine the prognostic value of short-term BPV on organ damage related to retinal microvascular abnormalities in a post-hoc, hypothesis generating analysis among 35 non-diabetic and 28 patients with type II diabetes mellitus (T2DM). The novel findings of this study were that the BPV–related mechanisms underlying microvascular complications may differ between people with and without T2DM.

Study 3 (chapter 3) sought to determine the prognostic value of short-term, mid-term and long-term BPV on organ damage related to heart structure and large artery stiffness in a follow-up study among 286 patients with uncomplicated hypertension and low to moderate cardiovascular risk. The important new findings were that changes in mean BP levels, but not

BPV, were most relevant to changes in organ damage in patients with uncomplicated hypertension. Therefore, BPV appears to offer limited clinical utility in this patient population.

Studies 4 and 5 aimed to determine the impact of within-visit BPV on hypertension diagnosis among adults (chapter 4), and children and adolescents (chapter 5), participating in the Australian Health Survey 2011-2013. Due to highly age-dependent reading-to-reading changes in BP, hypertension classification in adults varied according to the number of readings used for BP assessment (study 4). Moreover, the findings in study 5 showed that within-visit BP was highly variable in children and adolescents, with the magnitude of change being highly affected by BP level and age. The key finding from both studies was the significant impact of BPV on hypertension diagnosis, and this highlights the need for out-of-clinic BP measures to confirm diagnosis.

Study 6 (chapter 6), aimed to determine the effect of vitamin D supplementation on long-term BPV, aortic stiffness, peripheral and central BP indices, among 241 individuals with vitamin D deficiency and knee osteoarthritis. The results showed that vitamin D supplementation did not improve long-term BPV, aortic stiffness or any other BP indices, in this patient population. Following on from this work we were invited to write an expanded review on the evidence from published randomised controlled trials regarding the effect of vitamin D supplementation on cardiovascular surrogate and hard clinical endpoints, including BPV (Appendix 4; study 7). This review concluded that vitamin D supplementation was ineffective for improving cardiovascular health among various patient populations, including the presence or absence of vitamin D deficiency.

In summary, this PhD research program has made several novel observations. The methodology to quantify BPV can affect the magnitude of BPV itself and therefore BPV methodologies need to be standardized. Furthermore, the research has showed that short-term BPV may play a role in the pathophysiology of microvasculature in patients with T2DM; however, BPV (short-term, mid-term or long-term) did not offer additional prognostic value regarding organ damage (heart structure and large artery stiffness), over and above mean BP levels, among patients with uncomplicated hypertension and low to moderate cardiovascular risk. Additionally, within-visit BP was highly variable in adults as well as children and adolescents, implying that assessment and diagnosis of elevated BP should be confirmed using out-of-clinic BP monitoring. Finally, vitamin D was not effective in improving long-term BPV, large artery stiffness or other BP measures. Taken all together, this research provides novel

data that significantly adds to the current knowledge body around BPV, by addressing key literature gaps related to the measurement and clinical implications of BPV.

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Preface

High blood pressure (BP) is associated with cardiovascular morbidity and mortality; however, the exact underlying mechanisms are not clear. Whilst cardiovascular risk related to hypertension is traditionally assessed using the mean of clinic or out-of-clinic BP measurements, such as 24-hour or home BP, research has been exploring an alternative hypothesis which could offer important clinical information to the overall assessment of cardiovascular risk associated with high BP. This hypothesis suggests that assessing blood pressure by quantifying its fluctuations over time could add considerable clinically relevant information, over and above the mean BP level, and therefore could be a useful guide in the diagnosis and treatment treatment and diagnosis of hypertension.[1] Blood pressure variability (BPV) can be assessed from very short-term BP monitoring (measurements over minutes), short-term (measurements over a period of 24 hours), mid-term (measurements over days) and long-term (measurement over visits). Although, there has been an increasing number of studies exploring BPV in the last decade, significant research gaps remain.

Firstly, evidence from studies investigating BPV in relation to cardiovascular risk are equivocal and this may be due to the varying protocols and methodologies used to assess BPV. Although, there have been speculations that specific methodological factors could affect the magnitude of BPV itself (i.e. the number of BP readings used for assessment), no study has systematically explored the literature in order to identify specific methodological issues and provide recommendations for standardizing the protocols and methodology of measuring BPV. Moreover, different BPV measures (i.e. short-term or long-term) could be linked to different pathophysiological profiles[2] as well as different outcomes in specific populations, therefore the prognostic significance of BPV is still unclear. At the same time, the effect of very short-term BP fluctuations (over minutes or over readings) on hypertension classification and diagnosis,

in adults as well as children and adolescents, is unclear. Lastly, there are limited data from clinical trials that aim to reduce BPV via novel arterial destiffening interventions.

This research program comprises a series of analyses from convenience data samples, as well as systematic reviews, to address key deficits in the field of BPV. It was necessary to develop an understanding of the methodology used to quantify BPV and how this may affect the quantification of BPV. This was achieved using a systematic scoping review of the literature (chapter 1; study 1). Analyses conducted within chapters 2 and 3 were derived from two clinical studies completed within the Blood Pressure Research Group at Menzies, and enabled examination of key gaps related to the clinical implications of BPV, with end organ damage as the outcomes (retinal arteriolar markers, cardiac structure and large artery stiffness).

Chapters 4 and 5 were made possible from data publicly available within the Australian Health Survey 2011-2013. From these data we determined the clinical importance of BPV in relation to hypertension diagnosis in adults as well as children and adolescents. Lastly, to address a key gap related to interventions to modify BPV, we employed a retrospective analysis from within a randomised controlled trial where we aimed to investigate the effect of artery destiffening (via vitamin D supplementation) on long-term BPV. Confirmation of this analysis was then determined using a literature review which investigated the effect of vitamin D supplementation on cardiovascular clinical endpoints, including BPV.

The above aims were investigated in separate studies and have resulted in several published manuscripts, in addition to some manuscripts being prepared for publication or currently in submission, to peer-reviewed scientific journals. Published manuscripts or those which are being prepared for publication at the time of thesis submission are presented in their final submitted format as per the requirements of each journal; therefore, the presentation format and style of each individual chapter may be slight different between chapters. This thesis

contains 6 chapters and each chapter represents a separate study which directly contributes to the overall thesis aims. The individual contributions of each study to the thesis aims are presented at the end of each thesis chapter.

Chapter 1

Methodological factors affecting quantification of blood pressure variability: a scoping review

At the time of thesis submission, this chapter is under peer-review with the Journal of Hypertension.

Veloudi P., Sharman JE.

1.1 Abstract

Objectives. Blood pressure variability (BPV) may offer independent prognostic information. However, data supporting this notion are inconsistent, and this may be due to the wide variety of methodologies used for measuring BPV. A systematic analysis on the effect of BPV methodologies on BPV itself has never been undertaken and was the aim of this study. We also sought to determine the effect of participant characteristics on BPV.

Methods. A scoping review process was used to identify the effect of BPV methodologies on BPV magnitude. Key methodological factors assessed were 1) the number of BP readings or visits used to quantify BPV and 2) the duration of BP monitoring. Additionally, the relationship between age and mean BP level on BPV was investigated. Analyses were performed across studies that measured BPV over the short-term (using 24-hour BP), mid-term (using home BP in the morning, evening or day-to-day) and long-term (using visit-to-visit clinic BP).

Results. Data were extracted from 96 studies. The number of BP readings and visits used to quantify BPV were positively associated with mid- and long-term BPV. Duration was weakly associated with mid-term (morning) BPV. Age was positively associated with long- and mid-term (day-to-day), but not short-term BPV. Mean BP level was positively associated with BPV, except mid-term BPV (morning and evening).

Conclusions. The methodology used to quantify BPV, as well as age and mean BP level, affects the magnitude of BPV itself. This highlights the need to standardize BPV protocols, particularly regarding the number of BP readings and visits.

1.2 Introduction

A plethora of data suggest that blood pressure variability (BPV) may offer prognostic information, over and above mean BP levels.[2-5] Blood pressure (BP) is characterised by a degree of variability which can be observed over readings from minute to minute, hours, days, months or years. Three distinctive types of BPV have emerged among clinical studies during the last decade: short-term (using 24-hour BP monitoring) which quantifies BP fluctuations over hours; mid-term (using home BP monitoring) which quantifies BP fluctuations over days and lastly, long-term BPV (using clinic BP monitoring) which quantifies BP fluctuations over visits spaced over months or years. However, various protocols and methodologies have been used to quantify BPV, which could impact the magnitude of BPV itself. Additionally, it is not clear whether participant characteristics, such as age or mean BP level, could affect the magnitude of BPV and, therefore, the identification of normative reference ranges and cut-off values for risk stratification. Altogether, the above issues have created an uncertainty as to how BPV may be used in clinical practice.

Among the most important methodological issues that may affect the quantification of BPV is the number of readings used to quantify short- and mid-term BPV or the number of visits used to quantify long-term BPV, which vary greatly among studies. Another methodological issue is the duration of monitoring, in which various protocols have been used for mid-term and long-term quantification of BPV. For example, to quantify mid-term BPV, Fukui et al.[6] used 3 readings over 14 days, whereas Satoh et al.[7] used 28 readings over 28 days. On the other hand, for the quantification of long-term BPV, Sohn et al.[8] used 6 visits over 3.5 years, whereas Chia et al.[9] used 3 to 4 visits per year, over 15 years of BP monitoring. Moreover, it has been suggested that BPV magnitude may be affected by participant characteristics such as age and mean BP level. Although, methodological discrepancies concerning the measurement of BPV have been noted by many,[10,11] there has never been a systematic review of the literature to

quantify the effect of the number of BP readings or the duration of BP monitoring used to quantify BPV, nor the effect of participant characteristics, on BPV assessment. Therefore, this study aimed to identify how different BPV methodologies may affect the magnitude of BPV with the following foci: 1) the number of BP readings or visits used to quantify BPV and 2) the duration of BP monitoring. Additionally, this analysis sought to investigate the relationship between age and mean BP level on BPV magnitude.

1.3 Methods

Scoping review process

A scoping review technique was chosen as the most appropriate method to address the aims.[12] This involved an iterative approach to examine and describe the literature, which was based on Arksey and O'Malley's five-stage scoping review process:[13] 1) identify the research question, 2) identify relevant studies, 3) select the studies, 4) chart the data and 5) report the results. Following the identification of the main research question (already described in the introduction as the study aims), the literature was examined to identify the most common metric used to quantify BPV, and the last step was identification of studies that had reported average BPV for the population under investigation.

Publication of studies examining BPV date back to the late 1920s;[14] since then, an abundance of studies examining mainly short-term BPV have been published. However, a series of key-papers on BPV by Rothwell et al. published in 2010,[1,15,16] was a turning point towards publication of studies that sought to examine BPV using a variety of BP monitoring techniques (long-term, mid-term BPV). In view of the changing nature of BPV research and clinical practice, this work was not intended to analyse all available data in the field of BPV, but moreover to restrict analysis to capture a representative sample of contemporary BPV studies in which short-, mid- and long-term BPV was measured, as per the intent of a scoping review.[12]

Therefore, the 2006-2016 timespan; a decade which depicts the range of contemporary methodological approaches in BPV research, was chosen to provide representative data. Studies were identified through an English language search of PubMed, google scholar and grey literature using the following keywords: short-term, ambulatory, mid-term, home, day-to-day, long-term, visit-to-visit and blood pressure variability or blood pressure fluctuations or blood pressure variations.

Study selection

The criteria for inclusion were defined based on an iterative analysis of the literature, as described above. Our initial goal was to determine the most common type of metric used to quantify BPV (e.g. standard deviation, SD; coefficient of variation, CV; average real variation, ARV etc.) and then concentrate our final data synthesis on those studies that reported average BPV using this common metric. All study designs were eligible for inclusion and, similarly, all ages of subjects and all type of subject populations were eligible for inclusion. We excluded studies examining very short-term BPV (from beat-to-beat BP measures or reading-to-reading within-visit BP measures) as well as reviews (narrative, systematic or meta-analyses).

Data synthesis and analysis

Short-term BPV. Short-term BPV was quantified from day or awake 24-hour BP monitoring. The number of readings and duration of monitoring were estimated based on the protocols described by each paper (i.e. if day BPV was monitored from 06:00 am to 22:00 pm using an interval of 20 minutes between each reading, the estimated number of readings would be 48 and the duration would be 16 hours).

Mid-term BPV. Mid-term BPV was quantified from either morning, evening or day-to-day home BP monitoring. The number of readings taken at each occasion from the participants at home, as well as the duration in days was used to calculate an estimated number of overall

readings used to quantify mid-term BPV (i.e. if participants were instructed to measure their BP once every morning for 28 days then the number of readings reported would be 28). For studies which have used the average of more than one BP reading to define each session (i.e. morning, evening or total day BP level), the number of readings was based on the total averaged BP for each session.

Long-term BPV. Long-term BPV was quantified by visit-to-visit clinic BP monitoring. The estimated total number of visits was calculated based on the protocol provided (i.e. if BP was measured in clinic 2 times per year and the duration of monitoring was 5 years, the number of visits would be 10). Alternatively, we used the mean number of visits if that was reported. As there was a substantial number of studies that reported BPV using both SD (n=48) and CV (n=26), the analysis was performed for both metrics.

Analysis. Studies were examined and the main themes were identified; type of BPV, type of metric used to quantify BPV, number of BP readings or visits, duration of BP monitoring, age and mean BP level of the population under investigation (step 1 as per Arksey and O'Malley).[13] Studies were then identified and selected based on the main themes (2nd and 3rd steps) and included in a data chart for further analysis (step 4). In order to construct an overall perspective and analytically interpret the evidence, the data were examined based on descriptive statistics (frequency, mean or range) and visually inspected using scatter plots and bar charts to identify relationships between the variables under examination (step 5).

1.4 Results

Literature review. A total of 269 studies on systolic BPV were examined; 110 studies examined short-term BPV, 33 studies examined mid-term BPV and 124 studies examined long-term BPV. The most common metric used across all studies was SD 74%, n=199), followed by the CV (43%, n=116) and ARV (22%, n=58), as seen in Figure 1.1 (metric segment**)**. 26

studies examining short-term BPV (day or awake BPV), 15 studies examining mid-term BPV (morning, evening or day-to-day BPV) and 55 studies examining long-term BPV (visit-to-visit BPV) reported mean BPV using SD (Table 1; supplementary material [Appendix 1]).

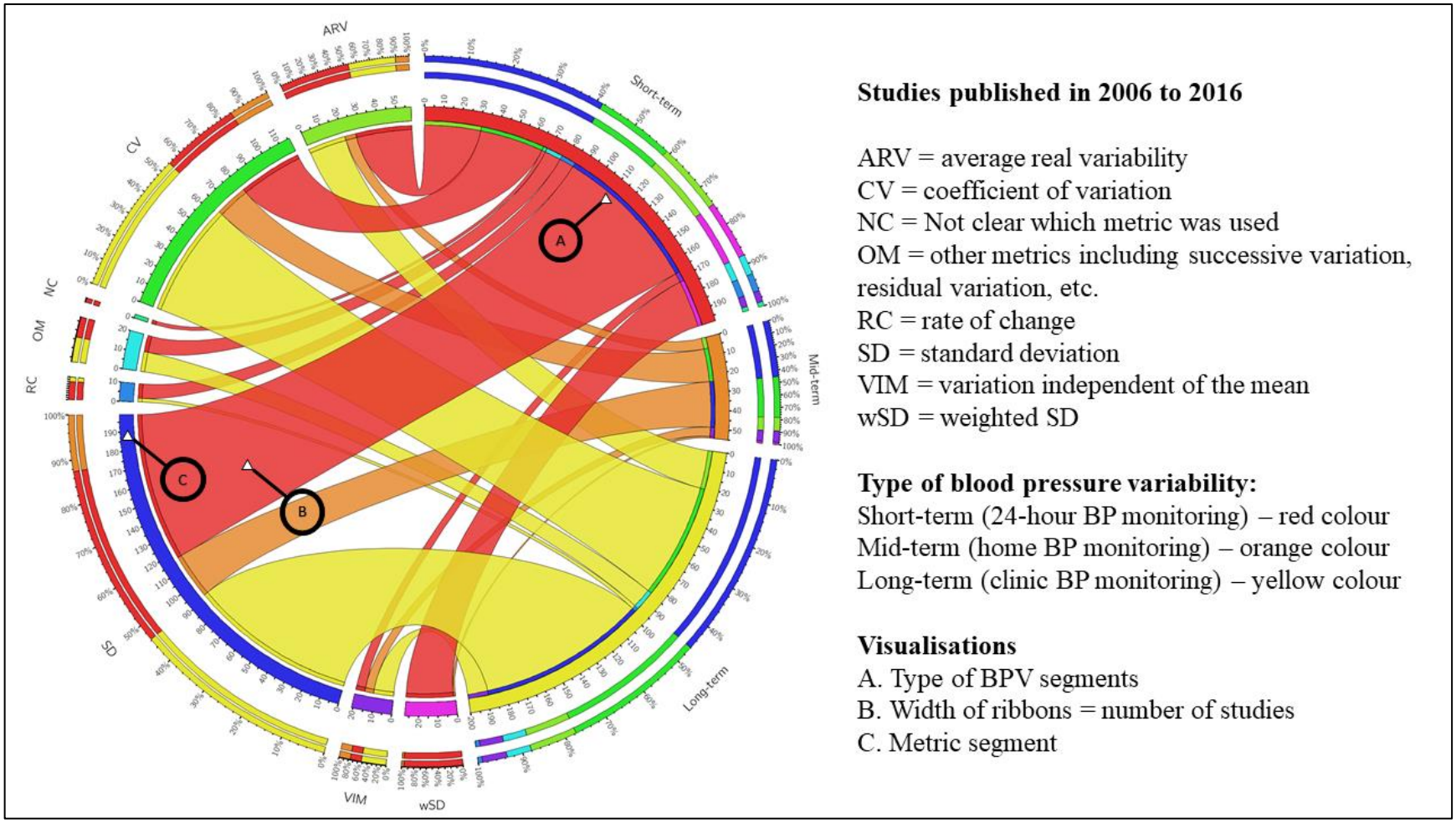

Figure 1. 1. Circular representation of the prevalence of various blood pressure variability (BPV) metrics across studies examining different types of BPV; short-term (red colour), mid-term (orange colour) and long-term (yellow colour).
The width of ribbons shows the number of studies which have used a certain metric under a certain type of BPV (i.e. 82 studies examining short-term BPV, 34 studies examining mid-term and 92 studies examining long-term BPV have used SD). Metric segment show the total studies which have used a specific metric, i.e. blue colour show studies which have used SD; n=199, green colour show studies that have used CV; n=116.

Short-term BPV. Table 1 (supplementary material [Appendix 1]) shows the various protocols used to determine the day or awake time periods; the majority of studies used fixed times whilst other studies used participants' diaries to identify day or awake period. Studies used the following time intervals between readings; 15 min, 20 min and 30 min, with the most common being a 30 min interval (n=10 from 26). The number of readings used to quantify short-term BPV ranged from 32 to 64. The duration of day-time monitoring period varied across studies with the longest monitoring being 17 hours and the shortest being 10 hours. Mean short-term BPV was 13.9 (2.9) mmHg. The magnitude of BPV was not associated with the number of BP readings or the duration of BP monitoring. Similarly, BPV was not associated with age, but it was positively associated with mean BP levels (Figure 1. 2).

Mid-term BPV. Mid-term BPV was separately assessed for morning (n=12), evening (n=7) and day-to-day BPV (n=7; Supplemental digital content; table 1). The number of readings used across studies ranged from 6 to 42 and the duration ranged from 4 to 28 days. Mean mid-term BPV was 8.2 (1.4) mmHg for morning, 8.3 (1.04) mmHg for evening and 8.6 (4.2) mmHg for day-to-day BPV. The number of readings as well as the duration of monitoring were positively associated with all types of mid-term BPV whilst age was positively associated only with day-to-day BPV (Figure 3). Although day-to-day BPV appeared be positively associated with mean BP levels, there was a weak negative association between mean BP level and morning or evening BPV.

Long-term BPV. The protocols used to quantify BPV are seen in Table 1 (supplementary material [Appendix 1]) and varied in terms of number of readings (range from 2 to 46 readings) and duration of monitoring (range from 0.2 to 25 years). Mean long-term BPV was 12.0 (5.1) mmHg calculated using SD and 9.5 (4.1) % calculated using CV. The number of visits was positively associated with long-term BPV whilst there was no association between the duration of monitoring and long-term BPV. Long-term BPV was also positively associated with age.

Lastly, long-term BPV, calculated as either SD or CV, was positively associated with mean BP level (Figure 1. 4).

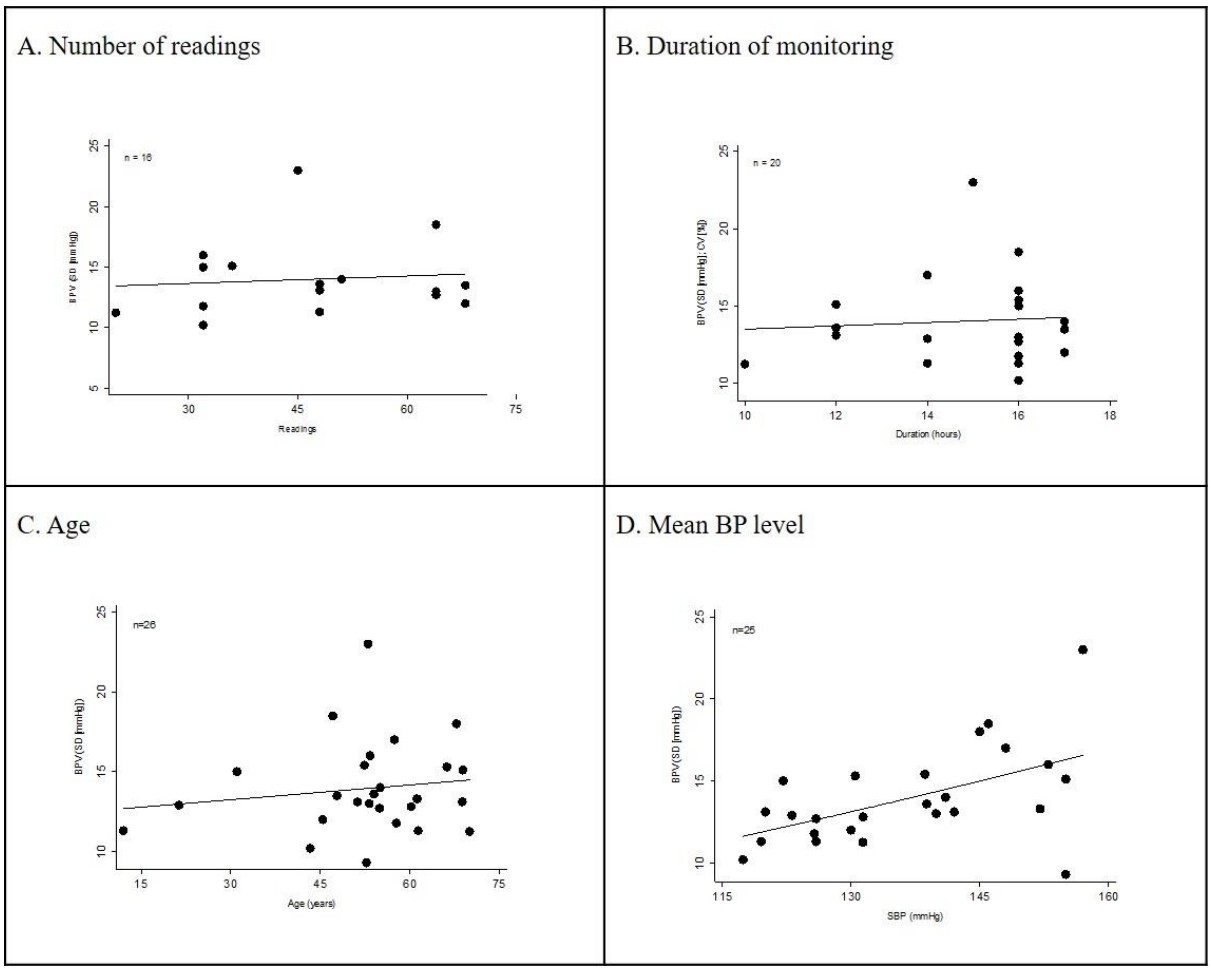

Figure 1. 2. Short-term BPV.
Scatter plots and fitted regression lines of the relationships between short-term (day or awake) systolic blood pressure variability and the number of blood pressure readings (panel a; r=0.10 p=0.72); duration of blood pressure monitoring (panel b; r=0.07 p=0.76); age (panel c; r=0.15 p=0.47); mean blood pressure level (panel d; r=0.53 p=0.01). Scatter plots represent data from individual studies.

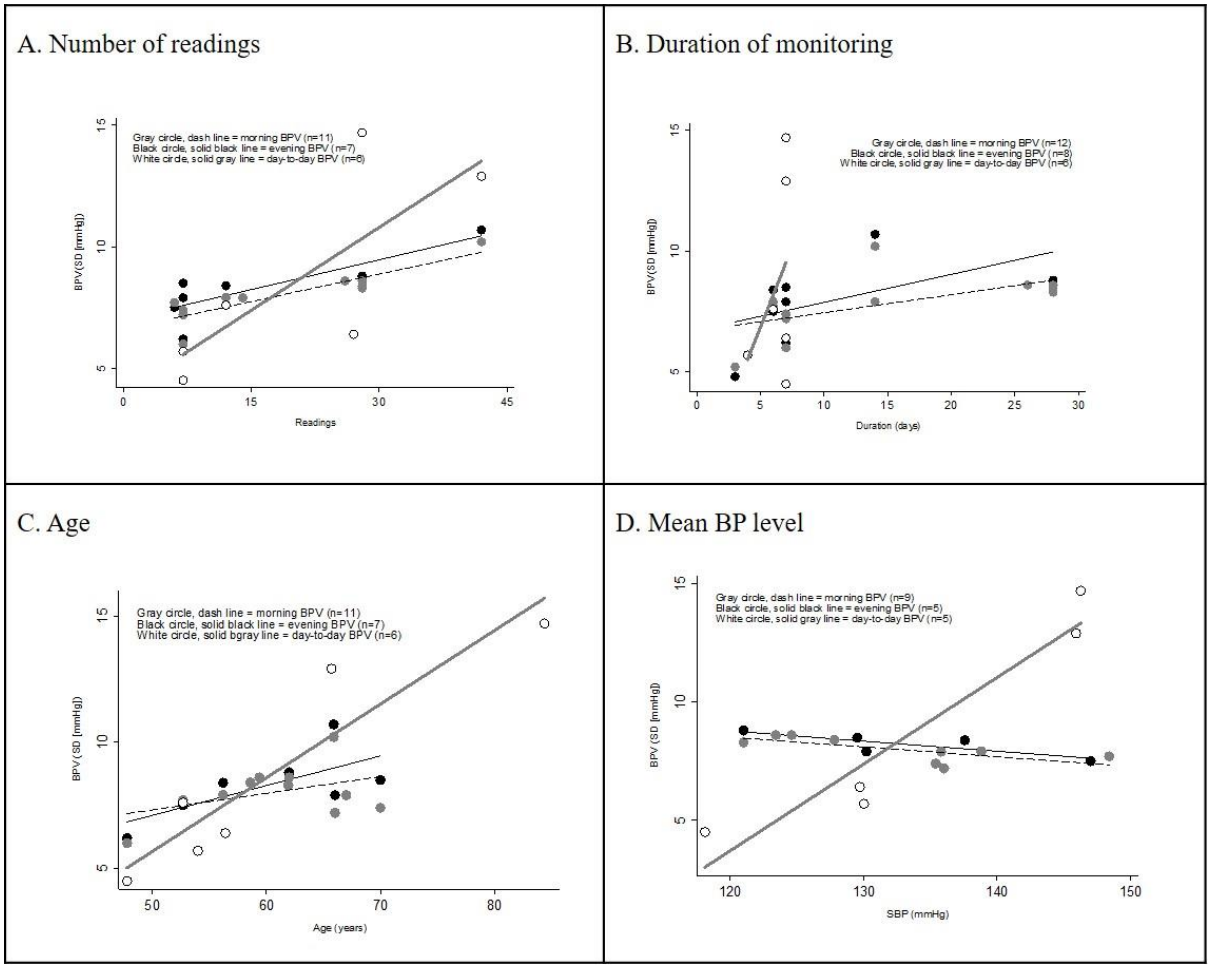

Figure 1. 3 Mid-term BPV.

Scatter plots and fitted regression lines of the relationship between mid-term systolic blood pressure variability (gray circles = morning; black circles = evening and white circles = day-to-day BPV) and the number of blood pressure readings (panel a [morning: r=0.10, p=0.018; evening: r=0.10, p=<0.001; day-to-day: r=0.23, p=0.07]); duration of blood pressure monitoring (panel b [morning: r=0.60, p=0.04; evening: r=0.52, p=0.18; day-to-day: r=0.39, p=0.44]); age (panel c [morning: r=0.07, p=0.20; evening: r=0.12, p=0.09; day-to-day: r=0.29, p=0.008]); mean blood pressure level (panel d [morning: r=-0.041, p=0.03; evening: r=-0.043, p=0.095; day-to-day: r=0.37, p=0.01]). Scatter plots represent data from individual studies.

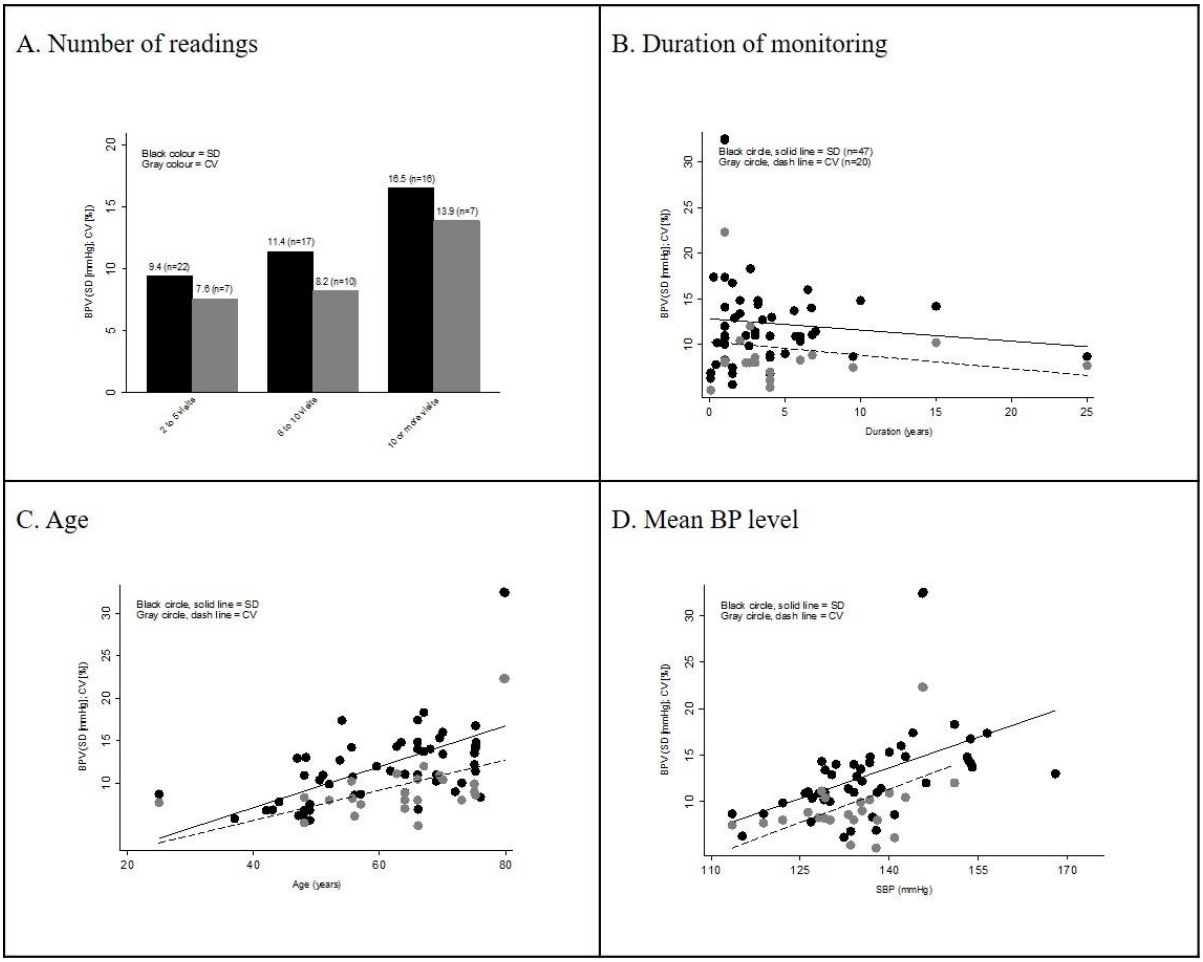

Figure 1. 4. Long-term BPV.
Scatter plots and fitted regression lines of the relationship between long-term systolic blood pressure variability (black circles = standard deviation [SD]; gray circles = coefficient of variation [CV]) and the number of blood pressure visits (panel a); duration of blood pressure monitoring (panel b [SD: r=-0.10, p=0.52; CV: r=-019, p=0.43]); age (panel c [SD: r=0.57, p<0.001; CV: r=0.52, p=0.01]) mean blood pressure level (panel d [SD: r=0.49, p=0.001; CV: r=0.49, p=0.02]). Scatter plots represent data from individual studies.

1.5 Discussion

Although, methodological discrepancies concerning the measurement of BPV have been noted by many,[10,11]suggesting that BPV magnitude may be affected by participant characteristics such as age and mean BP level, to our knowledge, this is the first systematic review of methodological factors that could affect the assessment and magnitude of short-, mid- and long-term BPV. The key findings were firstly, the number of BP readings used to quantify mid-term BPV and the number of visits used to quantify long-term BPV were positively associated with the magnitude of BPV itself. However, the number of BP readings was not associated with the

magnitude of short-term BPV. Secondly, the duration of BP monitoring was not associated with the magnitude of short- and long-term BPV although there was a weak association between the duration of BP monitoring and mid-term BPV. Lastly, long- and mid-term (day-to-day), but not short-term BPV, were positively associated with age, and importantly, most types of BPV were positively associated with mean BP levels. These findings indicate that the magnitude of BPV is not only dependent on several methodological factors, but also on participant age and mean BP levels. These data emphasise the need to standardize the methodology of measuring and quantifying BPV.

Short-term BPV. The methodology and protocols used to measure mean BP levels using 24-hour monitoring are well defined and usually require measurements to be taken every 20-30 minutes during daytime and every 30-60 minutes during night-time, with an interval of 15-30 minutes generally accepted for use throughout the entire 24-hour period.[17] It has been suggested though that the quantification of short-term BPV may require a higher frequency of measurements with time intervals ≤15 minutes,[2] but the exact time interval required for an accurate quantification is not clear. Importantly, Di Rienzo, using continuous intra-arterial BP monitoring and comparing with BP measurements taken every 5, 10, 15, 30 and 60 minutes, concluded that although mean BP levels were correctly quantified using 30-minute intervals, short-term BPV could still be erroneous even at sampling intervals of 5 and 10 minutes.[18]Our analysis showed that the number of readings used to quantify day or awake short-term BPV was not associated with the magnitude of BPV; however, none of the studies which met our inclusion criteria used a time interval <15 minutes. Although some guidelines recommend that 14 readings may be adequate to accurately estimate short-term BPV,[19] there is evidence supporting the need for higher frequency of BP sampling. Therefore, future studies examining short-term BPV should use a protocol with a sampling time-interval of <15 minutes until methodological studies establish a reliable and widely accepted measurement protocol.

Moreover, there is no standard definition as to what constitutes the 'daytime' period, and daytime short-term BPV has been quantified over periods that range from 10 to 17 hours. Similar to the number of readings, the duration does not seem to affect the magnitude of short-term BPV according to the results of this analysis. One of the main methodological issues reported for the quantification of short-term BPV, is the relationship of the SD to the mean BP levels,[20] and our analysis of studies confirms that SD increases as mean BP level increases. Although, an association between BPV and mean BP levels has been reported in the past, this is the first time that the strength of this association has been quantified. The findings of this study show that associations between BPV and mean BP levels ranged from as low as 0.04 to as high as 0.5 (quantified by Pearson correlation coefficient), depending on the type of BPV. The above highlight the need to keep in mind that a strong association between BPV and mean BP level might exist and could result in significant confounding even after adjustment in multivariable models. Lastly, short-term BPV is thought to increase with older age,[21] but there is minimal evidence to support this alleged relationship. Cross-sectional studies reported that short-term BPV is positively correlated with age;[22] yet contradictive results were reported in a 5-year follow up study of 162 healthy individuals, which found that short-term BPV decreased over time.[23] Even though, short-term BPV may appear to be correlated with older age in cross-sectional studies, this may be due to a higher mean BP level with older age; therefore it is important that interactions between age and mean BP level are explored in future studies.

Mid-term BPV. Similar to short-term BPV, there are no clear recommendations as to how many BP measurements or how long the duration of BP monitoring should be undertaken for an accurate assessment of mid-term BPV. Our analysis showed that both the number of BP readings as well as the duration of BP monitoring were positively associated with mid-term BPV. Therefore, it is important that these methodological factors are standardized in order for results to be generalizable. Another methodological issue concerns the timing of the

monitoring, whether it should be undertaken only in the morning, evening or all-day BP measures. Our analysis showed that the magnitude of BPV appeared to be similar when assessed using morning or evening BP measures, but BPV was slightly higher when all-day measures were used (day-to-day). Additionally, it has been suggested that mid-term BPV is affected by age and mean BP levels.[11] The results of this analysis confirm a positive association of day-to-day mid-term BPV though this was not the case for morning and evening mid-term BPV. Similarly, day-to-day BPV was positively associated with mean BP level, although paradoxically, morning and evening mid-term BPV appeared to be slightly decreasing with higher mean BP level.

The first paper to identify an outcome-driven cut-off value for mid-term BPV, as quantified by day-to-day home BP monitoring, was published recently by Juhanoja et al.[4] They concluded that a systolic BPV greater than 11.0%, measured using CV, indicates a higher cardiovascular risk in European and Asian general populations with a mean age of 60 and a mean BP level of 128.7 mmHg. The same study reported a primary cut-off value for BPV which was calculated using the first morning BP measurements for days 3 to 7 (11.0%) and also reported cut-off values which derived from sensitivity analysis using all 7 day measurements or the measurements of the first 3 days; 10.7% and 11.5% respectively. Lastly, the authors argued that the CV could be used as a universal reference metric due to low computational complexity (Table 1. 1) enabling easier calculation in clinical practice. We found that the most common metric used in research is the SD, followed by the CV. The reason of SD and CV being the most commonly used metrics for the quantification of BPV could indeed be their low computational complexity, but it needs to be confirmed if these metrics are interchangeable as markers of prognosis.

Table 1. 1. Metrics used in blood pressure variability quantification

Metric	Formula	Computational complexity	Characteristic
Standard deviation (SD)*	$\sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$	Low	Reflects the dispersion of values around the mean; it may be correlated with mean BP level (higher BPV may be observed if mean BP levels are high and vice versa)
Coefficient of variation (CV)	$\frac{SD}{\mathrm{x}} \times 100$	Low	Reflects the dispersion of values around the mean but it takes into account the level of mean BP. Using CV one could compare the variability in measures even when the means are different. However, CV may retain an association with mean BP level, although it may be weaker in comparison to SD
Rate of change (RC)	$\sum \frac{n-1\lvert r_i \rvert}{n-1}$ Where r is the rate of change between two readings and it is computed as described by Zakopoulos et al.[24]	High	Reflects the speed of change in BP and it shows how fast BP changes from reading to reading, day to day or visit to visit
Variation independent of the mean (VIM)	$\frac{SD}{\bar{x}^c} \times k$ Where c is the power at which the mean BP is raised to and derives from curve fitting and k is a constant, computed as described by Rothwell et al.[16]	High	This metric is a transformation of SD where the mean is raised to a certain power in order to remove any correlation between the SD and the mean BP level. This metric is therefore considered to be a BPV measure independent of the mean BP level
Average real variability (ARV)	$\frac{1}{n-1}\sum_{i=1}^{n-1}\lvert x_{i+1} - x_i \rvert$	Low	Accounts for the order in which BP readings were taken and it is a less sensitive metric of variance if BP readings are not frequent

*Standard deviation can be quantified by weighting the different time periods (i.e. weighted for daytime and night-time periods for a 24-hour quantification of BP.

Long-term BPV. As it has been noted by Whittle in a recent editorial,[25] long-term BPV was more powerful in terms of prediction of cardiovascular disease[26] and events such as stroke and coronary events[1,27] in comparison to other BPV monitoring techniques (i.e. short-term BPV). Despite the plethora of studies investigating the effect of long-term BPV on outcomes, there is no consensus as to the protocol for BPV assessment regarding the number of visits or the duration of BP monitoring[10] and importantly, there are no cut-off values for risk stratification. The above issues constitute important barriers in the incorporation of long-term BPV in clinical practice.

It has been reported that the number of visits used to quantify long-term BPV is positively related to BPV magnitude.[28] Levitan et al. has also reported that the duration of BP monitoring as quantified by the time-interval between visits may impact BPV quantification. Our analysis confirms that the magnitude of long-term BPV increased as the number of visits increased. On the other hand, our study found that the duration of BP monitoring was not associated with the magnitude of long-term BPV. Although this might seem contradictory, it is important to note that a longer duration of BP monitoring does not necessarily mean a higher number of BP visits or readings (a 10-year study might have quantified BPV using BP readings from 3 visits whilst a 6-month study might have quantified BPV using BP readings from 6 visits). Levitan et al, showed that BPV quantified using 7 visits over 18 months was 6.8 mmHg and 7.5 mmHg when the time interval was spaced across 4 years; however, BPV was 7.7 mmHg when BPV was quantified using 18 visits and 4 years BP monitoring duration. However, the difference between BPV over 4 years quantified by 7 versus 18 visits was not statistically significant. Moreover, it is not clear whether and how age and mean BP level could affect the assessment of long-term BPV. Our results showed that both SD and CV of long-term BPV were positively associated with age and mean BP levels.

Some studies have recommended long-term BPV cut-off values denoting the development of renal functional decline[9] and chronic kidney disease;[29] 13.5 mmHg and 14.8 mmHg, respectively (both using the SD). These values derived from 3 to 4 visits every year, over a 15 year follow up duration as reported by Chia et al.[9] and from 12 visits as reported by Yokota et al.[29] (the duration of BP monitoring was not reported in this study). In the latter study, subjects were older by a decade (67 versus 56 years old) which could have also accounted for the difference observed, on the basis of our findings that age was positively associated with the SD in long-term BPV. On the other hand, Kawai et al.[30] reported that a BPV of 8.3 – 8.4 mmHg (SD) could predict cardiovascular events and a BPV of 13.7 mmHg could predict total mortality. BPV in that study was quantified from 6 visits, in a hospital-based cohort with a mean age of 62 years. Based on the findings of this current analysis, none of the cut-off values reported above are comparable due to the heterogeneity of the methodology used to quantify BPV as well as the different population characteristics (i.e. different age).

Strengths and limitations. The scoping review approach enabled, for the first time, to evaluate and quantify the effect of certain methodological factors of BPV assessment on the magnitude of BPV itself. On the other hand, the analysis was restricted to studies reporting BPV as quantified by SD; this restriction was necessary due to the volume of BPV-related published studies and the various metrics used. Therefore, it could be argued that the results may not applicable to other measures such as VIM or ARV; however, given that most of the studies did not report average BPV and that SD is still the most common metric used in research, we believe that this analysis is a reasonable representation of current practice. Nonetheless, given that previous research has shown that there is a strong correlation (>0.95)[31] between SD and other BPV indexes, these results have important implications irrespective of the index of BPV.

Conclusion and recommendations. In conclusion, the methodology used to quantify BPV, as well as age and mean BP level, affects the magnitude of BPV itself. The implications of

methodological heterogeneity in measuring BPV as well as data reporting are critical in answering the questions of whether BPV and which type of BPV, independently predicts outcomes and whether it could be used in risk stratification. Nonetheless, what will ultimately guide clinical practice is universal thresholds for each type of BPV that will allow clinicians to interpret a patient's BPV according to certain reference values. Taken altogether, this study highlights the need for recommendations for the measurement of BPV, firstly regarding methodological factors underlying disparate values and secondly, regarding the type of data that should be reported. These recommendations should be followed by large-scale population studies that will include the full age and BP spectrum in order to quantify reference ranges and cut-off values for risk stratification. We suggest the following: 1) the timing, number of BP measurements and duration of BP monitoring should be clearly reported in all studies, 2) as the best definition of BPV remains to be established, a metric which has low computational complexity (i.e. CV) should always be reported to enable easier translation of results and integration in clinical practice, even if other independent of the mean metrics are the primary focus of analysis, 3) the statistical analysis should always take into account the effect of age and mean BP level by adjusting for these variables and especially, by exploring interactions between age and BP level.

1.6 Contribution of chapter 1 to thesis aims

Chapter 1 (study 1) was primarily used to review the literature and chart current evidence regarding the methodology used to quantify BPV. The results of chapter 1 represent the first scoping review of contemporary methodologies used to assess and measure the three main BPV types; short-term, mid-term and long-term BPV and it is the first study to identify methodological factors that could affect BPV magnitude. Importantly, it has been demonstrated that the number of BP measurements or visits used to quantify BPV, as well as the duration of BP monitoring, could affect the magnitude of BPV itself. Additionally, this study showed that

age and mean BP level are important determinants of the magnitude of BPV. These results highlight the need to standardize BPV protocols, particularly regarding the number of BP readings and visits.

Chapter 2

Associations of blood pressure variability and retinal arteriolar diameter in participants with type 2 diabetes

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2.1 Abstract

Blood pressure variability (BPV) is associated with macrovascular complications and stroke, but its association with the microcirculation in type II diabetes (T2DM) has not been assessed. This study aimed to determine the relationship between BPV indices and retinal arteriolar diameter in non-diabetic and T2DM participants. Digitized retinal images were analysed to quantify arteriolar diameters in 35 non-diabetic (aged 52[11] years; 49% male) and 28 T2DM (aged 61[9] years; 50% male) participants. BPV was derived from 24-hour ambulatory BP. Arteriolar diameter was positively associated with daytime rate of systolic BP variation (p=0.04) among T2DM participants and negatively among non-diabetics (p=0.008) (interaction p=0.001). This finding was maintained after adjusting for age, sex, body mass index and mean daytime SBP. These findings suggest that the BPV-related mechanisms underlying retinal vascular disease may differ between people with and without T2DM.

2.2 Introduction

Quantitative measures of retinal vascular structure, such as vessel diameters may provide prognostic information regarding microvascular complications and risk related to vascular diseases such as type II diabetes (T2DM). Retinal microvascular changes have been related to hypertension[32] whilst changes in retinal arteriolar diameters could be an early pathophysiological indicator in T2DM.[33] Although narrower retinal arteriolar diameters have been found to be associated with high blood pressure (BP),[34] possibly indicating vasoconstriction or inward remodelling in response to elevated BP, wider retinal arteriolar diameters were found to be associated with T2DM,[35] suggesting impaired arteriolar autoregulation[36] due to compromised myogenic responses. Negative correlations between BP and arteriolar diameters have also been observed in T2DM,[37] but evidence suggests these relationships may be weaker than in non-diabetic individuals.[38]

On the other hand, BP variability (BPV) indices such as the rate of BP variation over time (a measure of the speed of BP change), is thought to be an indication of the alterations in cardiovascular regulatory mechanisms that could lead to vascular complications irrespective of the mean (average) BP level.[39] At the same time, increased arterial stiffness and autonomic dysregulation, which could both lead to increased BPV, are common complications in T2DM.[40,41] Nevertheless, the relevance of BPV or mean BP level to retinal arteriolar diameters as derived from 24-hour ambulatory BP has never been investigated in individuals with T2DM. The aim of this study was to examine the relationship between mean BP levels as well as BPV with arteriolar diameters, in individuals with T2DM. We hypothesized that BPV indices may play a role in retinal vascular structure, as assessed by the quantification of arteriolar diameter, in patients with T2DM.

2.3 Methods

Data from this study was derived from a previously published investigation among 80 consecutive participants with (n=40) and without T2DM (n=40) recruited from the local community.[42] This current post-hoc, hypothesis-generating analysis was from the 35 non-diabetic and 28 T2DM participants where both retinal imaging and 24-hour ambulatory BP measurements were available. Exclusion criteria included pregnancy, arrhythmia or a clinical history of cardiovascular disease (including coronary artery disease, myocardial infarction, heart failure or stroke) or severe pulmonary disease. T2DM was self-reported after diagnosis by a doctor. The Human Research Ethics Committee of the University of Tasmania approved the study and written informed consent was provided by all participants.

Retinal imaging. Retinal images were recorded using a non-mydriatic retinal camera (Canon, CR-DGi-45NM) and digitised to a resolution of 2800-2400 pixels using a LS100 slide scanner (Nikon, Tokyo, Japan). Microvascular parameters were measured using a custom written Matlab program, as described previously.[43] Good reproducibility of this technique has been previously reported.[43] The analysis was performed on a series of complete vascular branches, either from one eye or both eyes in order to obtain the required number of vessel segments (8, range 7-18) and bifurcations (6, range 3-15). Arteriolar diameter was measured in a series of intensity cross-sections normal to the vessel at 2-pixel intervals along the entire length of the vessel segment. At each cross-section, arteriolar diameter was measured to subpixel accuracy using a sliding linear regression filter technique as described previously[43] and the average was calculated for each vessel.

24-hour ambulatory BP and BPV indices. 24-hour ambulatory BP was measured every 20 minutes during the daytime and every 30 minutes during the night-time (TM-2430, A&D Medical, Sydney, Australia). Participants were asked to continue their usual daily activities but to avoid any strenuous activities. Individual measurements with an error code or those with a pulse pressure of less than 20 mmHg were excluded from the analysis. BPV was expressed

separately for the periods of daytime, nighttime and 24-hour as the rate of BP variation and was calculated based on a modified method described by Zakopoulos et al.[44] Rate of BP variation was further adjusted for the corresponding mean BP levels ([rate of BP variation/mean BP]*100). Calculations involved plotting the slope for the change between SBP and DBP readings against time. Daytime was defined as the period between 08:00 to 20:00 and night-time as the period between 22:00 to 06:00. The periods from 20:00-22:00 and 06:00-08:00 were excluded as they represent the steepest diurnal fall and rise in BP respectively and, therefore may contribute to BPV measures. BPV was also quantified using the coefficient of variation of the corresponding mean BP level (CV; [standard deviation/mean BP]*100).

Blood biochemistry. Venous blood samples were drawn from the antecubital fossa and analytical biochemistry was performed by the Royal Hobart Hospital pathology department using accredited laboratory techniques.

Statistical analysis. Student's t tests were used to compare retinal and haemodynamic variables between diabetic and non-diabetic participants. Linear regression analysis was performed to assess the relationship between arteriolar diameter and BP measures, further statistical adjustment for possible confounders: age, sex, BMI (and mean SBP for BPV) was performed by multivariable linear regression. Statistical interactions between diabetes status and BP variables were assessed by testing the statistical significance of the coefficient of a product term after adjusted for age, body mass index (BMI), sex and mean daytime SBP (diabetes status x BP variable). A P value <0.05 was considered statistically significant. All data were analyzed using Stata 12.1 (StataCorp, College Station, Tx).

2.4 Results

Participants with T2DM were older, had higher BMI and most were on antihypertensive therapy or treatment with statins in comparison to non-diabetic participants (Table 2. 1). 24-hour ambulatory mean BP and BPV indices were similar between the groups (Table 2. 1) and

there were no differences in arteriolar diameters (non-diabetics; 25.54(2.74) pixels vs T2DM; 25.58(2.71) pixels, p=0.96). Retinal arteriolar diameter was not associated with any of the demographic characteristics (age, sex or BMI) among individuals with T2DM or individuals without T2DM (all p>0.09). Similarly, no associations were observed between retinal arteriolar dimeter and total cholesterol, triglycerides or glucose for individuals with T2DM or individuals without T2DM (all p>0.12).

Table 2. 1 Demographic and clinical characteristics of study participants.

Variable	**Non-diabetic (n=35)**	**T2DM (n=28)**	**P value**
Sex (male)	17(49)	14(50)	0.91
Age (years)	52(11)	61(9)	0.002
Body mass index (kg/m^2)	24.74(3.12)	31.71(5.55)	<0.001
Current smoker	3(8)	2(7)	0.67
Diabetes duration (years)	-	7(7)	-
Cholesterol (mmol/L)			
Total	5.37(1.02)	4.42(1.10)	0.001
High density lipoprotein	1.66(0.41)	1.30(0.46)	0.003
Triglycerides	0.93(0.46)	1.61(0.81)	0.0002
Glucose (mmol/L)	4.71(0.40)	7.65(1.89)	<0.001
Insulin (mU/L)	2.29(4.61)	10.00(8.62)	<0.001
Glycated haemoglobin (%)	5.52(0.36)	7.16(0.92)	<0.001
Medications			
Antihypertensive medications	0(0)	19(67)	<0.001
Statin	0(0)	19(67)	<0.001
Clinic BP (mmHg)			
Systolic blood pressure	113(9)	123(13)	<0.001
Diastolic blood pressure	65(6)	70(7)	0.004
24-hour ambulatory BP (mmHg)			
Daytime systolic BP	135(13)	138(13)	0.27
Night-time systolic BP	117(10)	122(3)	0.06
24-hour systolic BP	128(11)	132(12)	0.25
Daytime diastolic BP	84(7)	78(2)	0.008
Night-time diastolic BP	69(7)	69(8)	0.94
24-hour diastolic BP	78(6)	75(8)	0.07
Blood pressure variability (%)			
Daytime rate of systolic BP variation	23.97(4.58)	25.96(5.62)	0.13
Night-time rate of systolic BP variation	20.31(5.88)	18.83(5.77	0.32

24-hour rate of systolic BP variation	23.47(3.12)	25.09(5.35)	0.14
Daytime rate of diastolic BP variation	29.10(8.54)	29.58(6.75)	0.81
Night-time rate of diastolic BP variation	22.71(6.89)	24.49(8.16)	0.35
24-hour rate of diastolic BP variation	28.14(6.18)	29.21(5.89)	0.49

Data are mean(sd) or numbers (%). BP = blood pressure.

In univariate regression analysis, arteriolar diameter was associated positively with daytime rate of SBP variation among participants with T2DM (β=0.007; p=0.04; R^2=0.12) and negatively among non-diabetics (β=-0.010; p=0.008; R^2=0.17). Additionally, mean daytime SBP was negatively associated with arteriolar diameter among participants with T2DM (β=-0.003; p=0.03, R^2=0.16) but not among non-diabetics (β=0.0003; p=0.80). A significant interaction between diabetes status and daytime rate of SBP variation was observed and remained significant after adjusting for age, sex, BMI and mean daytime SBP (β=0.02; p=0.001) (Figure 2. 1). Moreover, the associations between arteriolar diameter and BPV were examined using other BPV indexes and measures that could derive from a short-term (24-hour) BP monitoring (daytime, night-time and total 24-hour BPV; SD, CV, time-rate and other indexes). Similar associations were also observed when CV was used. However, there were no significant associations between night-time BPV and retinal arteriolar diameter although there were similar, but weaker associations between total 24-hour BPV and the outcomes. Lastly, non-linear associations between BP or BPV indexes and retinal arteriolar were observed.

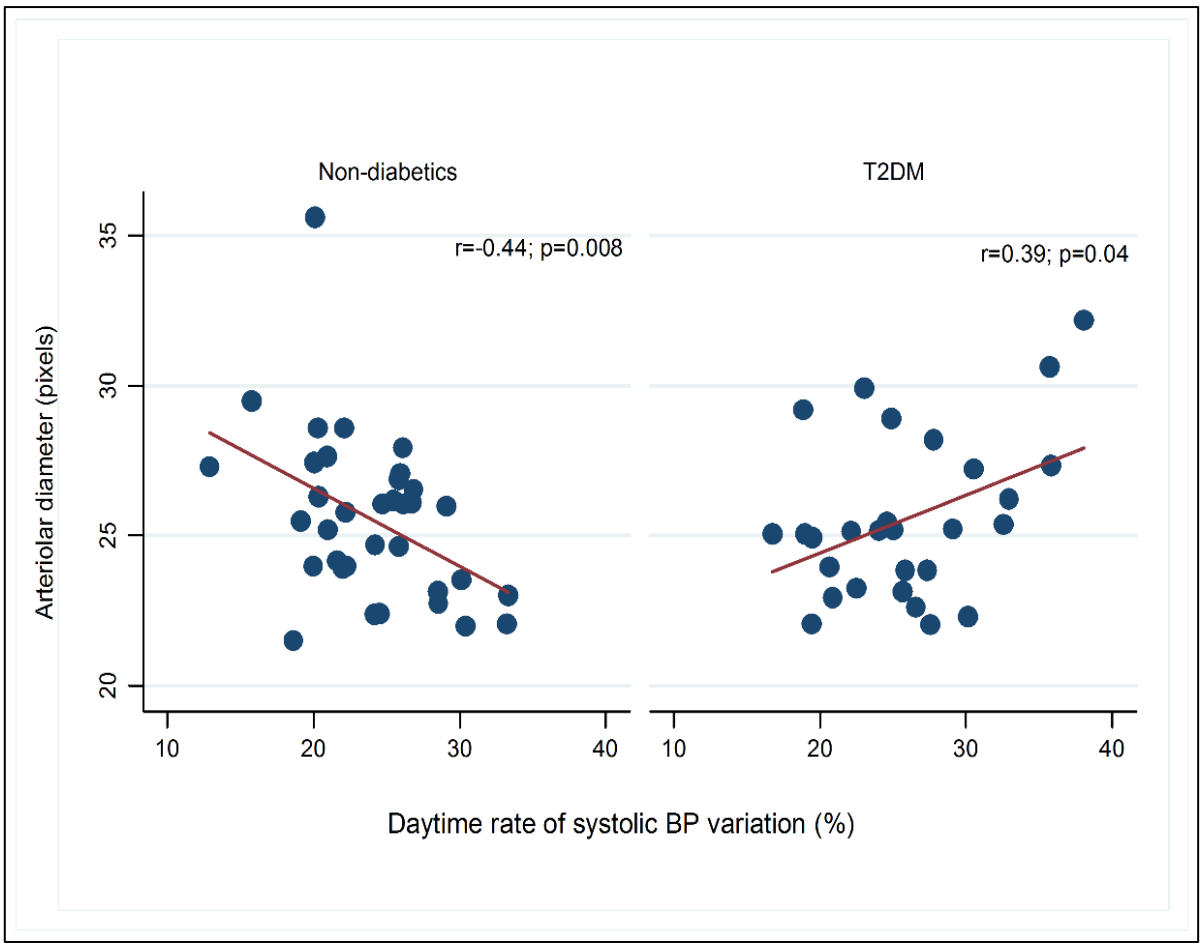

Figure 2. 1 Scatter plots and fitted regression lines of the relationship between arteriolar diameter and daytime rate of systolic BP variation among non-diabetics and participants with T2DM.

2.5 Discussion

The main finding of this study was that there were significantly different associations between retinal arteriolar diameter and BPV indices among healthy individuals and participants with T2DM. Retinal arteriolar diameter was positively associated with daytime SBP variation among participants with T2DM but negatively among non-diabetic individuals. These findings may suggest different pathophysiological contributions of BPV to adverse retinal arteriolar outcomes among people with T2DM compared with non-diabetic controls.

BPV could play a role in large artery damage and remodelling over and above mean BP levels; however, despite its importance in cardiovascular disease and stroke in particular,[39] the role of

BPV in microvascular disease is still unclear. This is the first study to assess the relationship between BPV and retinal arteriolar diameter and the impact of T2DM on that relationship.

Our findings of a positive relationship between BPV and retinal arteriolar diameter in participants with T2DM could be relevant to the mechanism or consequences of increased BPV. Arteriolar dilatation in association with higher BPV in T2DM could be an early sign of impaired vascular autoregulation[36] or early autonomic neuropathy.[41] Both are associated with increased risk of retinal microvascular damage that could result from exaggerated fluctuation in transmitted pressure or flow to the microcirculation. Additionally, the different associations between BPV and arteriolar diameter among the two groups may be explained by the different level of cardiovascular risk between the two groups (individuals with T2DM are at increased cardiovascular risk in comparison to those without T2DM). Some authors have suggested that associations between BPV and outcomes may be modified by the level of cardiovascular risk.[26,39]

These results should be seen in the context of the limitations of this study; the small sample size and the retrospective, cross-sectional design which limit the interpretation of the findings. The small size of our sample population may underlie the lack of stronger association between BP indices and arteriolar diameters. Indeed, further studies, in larger populations, are needed to examine associations between central haemodynamics, BP variability parameters and retinal arteriolar diameter in patients with T2DM. Similarly, a longitudinal follow-up study could help in determining causal relationships among haemodynamic parameters and retinal arteriolar diameter in diabetic microvascular disease.

In conclusion, the findings suggest that increased BP fluctuations may be associated with different arteriolar responses in comparison to elevated mean BP levels, with BPV possibly playing a role in the pathophysiology of retinal microvasculature in T2DM. Although the

mechanisms underlying the relationship between BPV and retinal arteriolar in T2DM remain unclear, our findings support the need for further investigation.

2.6 Contribution of chapter 2 to thesis aims

Chapter 2 (study 2) represents the first study that used short-term BPV indices as a means to try and help explain microvascular complications in people with type II diabetes mellitus. Although it was a small post-hoc study, it is the first hypothesis-generating analysis to offer evidence that increased short-term BPV may play a role in the pathophysiology of retinal microvasculature in type II diabetes mellitus.

Chapter 3

Pressure variability and prediction of target organ damage in patients with uncomplicated hypertension

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3.1 Abstract

Background: The average of multiple blood pressure (BP) readings (mean BP) independently predicts target organ damage (TOD). Observational studies have also shown an independent relationship between BP variability (BPV) and TOD, but there is limited longitudinal data. This study aimed to determine the effects of changes in mean BP levels compared with BPV on left ventricular mass index (LVMI) and aortic pulse wave velocity (aPWV).

Methods: Mean BP levels (research-protocol clinic BP [clinic BP], 24-hour ambulatory BP and 7-day home BP) and BPV were assessed in 286 patients with uncomplicated hypertension (mean age 64 ± 8 SD years, 53% women) over 12 months. Reading-to-reading BPV (from 24-hour ambulatory BP) and day-to-day BPV (from 7-day home BP) were assessed at baseline and 12 months, and visit-to-visit BPV (clinic BP) was assessed from 5 visits over 12 months. LVMI was measured by 3D echocardiography and aPWV with applanation tonometry.

Results: The strongest predictors of the changes in LVMI (ΔLVMI) were the changes in mean 24-hour systolic BPs ($p<0.02$). Similarly, the strongest predictors of the changes in aPWV (ΔaPWV) were the changes in mean 24-hour ambulatory systolic BPs ($p<0.01$) and the changes in mean clinic systolic BP ($p<0.001$). However, none of the changes in BPV were independently associated with ΔLVMI or ΔaPWV ($p>0.05$ for all).

Conclusions: Changes in mean BP levels, but not BPV, were most relevant to changes in TOD in patients with uncomplicated hypertension. Thus, from this point of view, BPV appears to have limited clinical utility in this patient population.

3.2 Introduction

It is well recognised that hypertensive target organ damage (TOD) and cardiovascular events are associated with elevated blood pressure (BP), as determined from the average of multiple BP readings (mean BP).[45] The extent to which BP fluctuates over time, expressed as blood pressure variability (BPV), has been suggested to offer incremental prognostic value, over and above mean BP levels.[1] However, the independent value of BPV is not yet established and results are conflicting between the different ways to assess BPV such as reading-to-reading, day-to-day or visit-to-visit BPV.[11,46-50] This lack of clarity with respect to the clinical significance of different BPV assessment methods may be related to the different pathophysiological pathways reflected by each type of BPV.[51] Importantly, there has never been a longitudinal study to examine the relation of BPV with TOD (for example, cardiac structure or aortic stiffness) utilizing more than one way to assess BPV in the same population. Therefore, a cause and effect relationship between BPV and TOD has not yet been clearly determined. On the contrary, the prognostic relevance of mean BP level as derived from clinic, 24-hour ambulatory or home BP to TOD is well known.[52,53] This study aimed to investigate the relationship between the changes in mean BP levels and BPV indices as derived from reading-to-reading (from 24-hour ambulatory BP), day-to-day (from 7-day home BP) as well as visit-to-visit BP monitoring (clinic BP), on changes in TOD (assessed by left ventricular mass index [LVMI] and aortic pulse wave velocity [aPWV]) in patients with uncomplicated hypertension followed over 12 months. We hypothesized that changes in BPV will be associated with changes in TOD indices and that these associations will be independent of mean BP levels.

3.3 Methods

Study population. Data were analysed from 286 patients with uncomplicated hypertension who participated in the BP GUIDE study, the design of which has been previously published.[54] Exclusion and inclusion criteria are seen in the supplementary material (Appendix 2). Patients gave informed consent prior to any assessments, and each study site was granted ethics approval by their local Human Research Ethics Committee.

Study protocol. Participants attended the study sites on 5 occasions over 12 months, at 3-month intervals. Clinic and home BP was measured at all 5 occasions and 24-hour ambulatory BP, LVMI and aPWV measurements were performed at baseline and at the 12-month visit. Complete data for all BP variables, as well as LVMI and aPWV measures were available on 267 and 250 participants respectively. Antihypertensive therapy was assessed at all 5 visits and recommendations regarding medication titration were provided to each patient's doctor. Extensive details on the titration recommendations have been published elsewhere.[54]

Target organ damage. Real-time three dimensional imaging was performed using a matrix array transducer and left ventricular mass (LVM) measurement was measured using real-time 3-dimensional echocardiography which has greater accuracy and lower test-retest variation, compared to other measurement techniques.[55] LVMI derived after indexing LVM to height$^{2.7}$ according to guidelines.[55,56] Aortic stiffness was assessed using tonometry readings of carotid to femoral pulse wave velocity, according to guidelines[57] with the patient in a supine position (SphygmoCor 8.0, AtCor Medical, Sydney, NSW).

Clinic BP. The average of two BP measurements taken 1 minute apart was calculated after 10 minutes of rest, which results in lower average BP that is more clinically relevant than averaging BP after 5 minutes rest.[58] We used a validated[59] automatic device for this purpose (Omron HEM-907; OMRON Europe B.V. (OMCE), Hoofddorp, The Netherlands). Measurements were recorded using an appropriate size of cuff, with the patients' arm supported at the height of the heart, back supported and feet flat on the floor as per recommendations.[45]

7-day home BP. A validated[60] oscillometric device (UA-767, A&D Mercury; A&D Medical, Thebarton, South Australia, Australia) was given to participants to record home BP. Participants were instructed to record their BP twice after 5 minutes of rest but only record the second reading. They were also instructed to take BP in the morning (between 06:00 and 10:00), in the evening (between 18:00 and 22:00) and at midday if possible.

24-hour ambulatory BP. A validated[61] BP device (TM-2430, A&D Mercury; A&D Medical) was used to take BP measurements every 30 minutes during the day and every hour during the night. The daytime period was defined as the time between 06:00 to 22:00 and the night-time period as the interval between 22:00 to 06:00. Participants were advised to maintain routine daily activities but to avoid strenuous physical activities. Data were included for analysis if there were more than ten BP measurements and if >80% of readings were valid. Measurements with an error code or a pulse pressure <20 mm Hg were excluded from the analysis.

BPV. Systolic BPV (SBPV) and diastolic BPV (DBPV) calculated as the standard deviation (SD) around the mean, as well as the coefficient of variation (CV; [SD/mean]x100), using the corresponding mean BP level. Reading-to-reading BPV derived from the 24-hour ambulatory BP monitoring and assessed separately for 24-hour, daytime and night-time periods. Day-to-day BPV was calculated from the 7-day home BP monitoring using all the available measurements rather than the average of each day in order to provide a greater number of BPs to allow for a better representation of BP fluctuations. Visit-to-visit BPV derived from clinic BP measured at 5 visits. The results are presented using the CV as it takes into account the relationship between mean BP levels and BPV.

Antihypertensive medications quantity. The daily defined dose (DDD) of medications was calculated as per World Health Organization standards and was recorded at all 5 visits; compliance was assessed by the study nurse viewing each participant's medication packet(s). Participants were categorised into groups based on the change in DDD over 12-months follow-

up; (1) those who had an increase, (2) those who had a decrease and (3) those who had no change, according to tertiles of the change in DDD. This enabled quantification of the change in DDD as well as the direction of the change (decrease or increase) over time. In this study, participants were randomised to have changes in medication guided by either usual care (based on clinic, 7-day, and 24-hour ambulatory BP) or additionally using central BP measures.[54]

Statistical analysis. The change in BPV and mean BP levels was compared between the upper and the lower quartile of ΔLVMI and aPWV in order to examine whether the respective change was different among those participants who had a sizeable change (increase versus decrease) in target organ damage. The relationships between the changes in LVMI (ΔLVMI) and aPWV (ΔaPWV) and the changes in mean BP levels or BPV measures were assessed using linear regression analysis which was repeated after adjusting for baseline age, sex and BMI. In order to assess if BPV indices were independently predicting changes in the outcomes, multivariable models were further adjusted for the changes in mean BP levels. Models were also adjusted for the change in DDD. Further sensitivity analyses are detailed in supplementary material (Appendix 2). Student's t-test was used to compare changes in mean BP and BPV variables between groups. A $p<0.05$ was considered statistically significant. Statistical analyses were performed using Stata 12.1 (StataCorp, College Station, Tx).

3.4 Results

Participant characteristics. Table 3. 1 shows the baseline demographic and clinical characteristics of the study participants. A small percentage of patients had type II diabetes (8%) whilst almost half of the study population were either current or former smokers. Mean LVMI was 31.28(5.54) $g/m^{2.7}$ and aPWV was 9.41 (2.14) m/s at baseline and values were not changed at 12-months on average; 31.30 (5.50) $g/m^{2.7}$ and 9.35 (2.02) m/s respectively. At baseline, the strongest predictor of LVMI was mean 24-hour ambulatory SBP ($p=0.03$) and clinic SBP for aPWV ($p<0.001$). None of the baseline BPV measures were correlated with

either LVMI or aPWV ($p \geq 0.08$ for all) whilst further adjustment for mean 24-hour ambulatory BP levels did not change these associations.

Table 3. 1 . Demographic and clinical characteristics of participants at baseline visit (n = 286)

Participant clinical characteristics	**Mean(SD) or %(n)**
Mean age, y	64(8)
Body mass index, kg/m^2	29(5)
Females	53 (152)
Smoking status (current or former)	44(126)
Type II diabetes mellitus	8(22)
Antihypertensive medications	
Angiotensin converting enzyme inhibitors	30(85)
Angiotensin receptor blockers	66(188)
Calcium channel blockers	30(84)
Diuretics	39(110)
Beta blockers	10(29)
Daily defined dose	2.4(1.3)
Blood pressure (mmHg)	
24-hour systolic blood pressure	131(12)
24-hour diastolic blood pressure	76(8)
7-day systolic blood pressure	128(13)
7-day diastolic blood pressure	74(8)
Clinic systolic blood pressure	127(14)
Clinic diastolic blood pressure	76(10)
Blood pressure variability (BPV; %)	
24-hour systolic BPV	16(5)
Daytime systolic BPV	15(5)
Night-time systolic BPV	13(5)
24-hour diastolic BPV	22(8)
Daytime diastolic BPV	20(8)
Night-time diastolic BPV	16(8)

7-day systolic BPV	7(2)
7-day diastolic BPV	8(2)

Associations of mean BP variables with ΔLVMI. Table 3. 2 shows the comparison of the change in mean BP levels between the upper and the lower quartile of ΔLVMI. All mean 24-hour ambulatory BPs indices, except mean night-time DBP were significantly increased among participants who had an increase in LVMI compared with those who had a decrease in LVMI. None of the changes in clinic or 7-day home BP were significantly different between the groups. In univariable analysis, ΔLVMI was positively associated with changes in mean 24-hour ambulatory, daytime and night-time SBP and remained significant in multivariable analyses adjusted for age, sex and BMI (Table 3. 3). No associations were observed between ΔLVMI and visit-to-visit mean BP (Table 3. 3). The above results remained unchanged after adjustment for changes in DDD.

Associations of BPV variables with ΔLVMI. 24-hour ambulatory and daytime SBPV were paradoxically decreased among participants who had an increase in LVMI compared with those who had decrease in LVMI (Table 3. 2). None of the changes in BPV measures were significantly different between the groups. Furthermore, a paradoxical, negative and independent association was observed between ΔLVMI and the changes in 24-hour SBPV but inclusion of the changes in mean night-time ambulatory SBP in the model rendered the relationship nonsignificant (p=0.14). None of the visit-to-visit BPV indices were associated with ΔLVMI (Table 3. 3). Results were unchanged after further adjustment for the changes in DDD.

Associations of mean BP variables with ΔaPWV. Table 3. 4 shows the comparison of the change in mean BP levels and BPV indices between the upper and the lower quartile of ΔaPWV. Mean 24-hour ambulatory, daytime SBP and DBP and mean clinic SBP and DBP

were significantly increased among participants who had an increase in aPWV compared with those who had a decrease in aPWV. None of the 7-day home BPs were significantly different between the groups. In univariable analysis, ΔaPWV was positively associated with changes in mean 24-hour ambulatory, daytime and night-time SBP; clinic SBP; and changes in mean 24-hour ambulatory, daytime, night-time and clinic DBP (Table 3. 3). All of these associations remained significant after adjusting for baseline age, sex and BMI (Table 3. 3); however, the associations between changes in mean clinic DBP indices and ΔaPWV were not significant after adjusting for changes in mean 24-hour ambulatory SBP or changes in mean clinic SBP ($p>0.05$ for all). Changes in mean visit-to-visit SBP and DBP were not associated with ΔaPWV (Table 3. 3). Results were unchanged after further adjustment for the changes in DDD.

Table 3. 2 Changes in mean BP levels and BPV indices among participants with a decrease or increase in LVMI over 12 months

Variable	**Decrease in LVMI (n=67)**	**Increase in LVMI (n=66)**	**P value**
24-hour ambulatory BP (mmHg)			
24-hour systolic BP	-1.03(13.10)	6.00(13.86)	0.01
Daytime systolic BP	-0.51(13.92)	5.29(13.44)	0.04
Night-time systolic BP	-1.63(14.63)	7.35(17.60)	0.006
24-hour diastolic BP	-0.43(8.58)	3.20(7.92)	0.03
Daytime diastolic BP	-0.63(8.34)	3.16(8.56)	0.03
Night-time diastolic BP	0.30(11.27)	3.35(9.50)	0.15
7-day home BP (mmHg)			
Systolic BP	1.08(10.33)	2.39(10.85)	0.49
Diastolic BP	1.37(6.73)	0.64(5.84)	0.51
Clinic BP (mmHg)			
Systolic BP	0.08(13.18)	0.18(13.97)	0.97
Diastolic BP	1.09(6.34)	1.63(5.73)	0.62
Reading-to-reading BPV (%)			
24-hour systolic BPV	0.51(3.37)	-2.07(4.16)	0.001
Daytime systolic BPV	0.61(4.13)	-2.02(4.01)	0.002
Night-time systolic BPV	-0.88(7.70)	-0.21(5.93)	0.63
24-hour diastolic BPV	0.22(6.97)	-1.25(7.29)	0.31
Daytime diastolic BPV	0.49(7.33)	-0.99(7.30)	0.31
Night-time diastolic BPV	-1.25(12.20)	-0.31(7.58)	0.65
Day-to-day BPV (%)			
Systolic BPV	-0.56(2.42)	-0.37(2.07)	0.64
Diastolic BPV	-0.50(3.13)	-0.50(3.12)	0.98

Data presented as mean(sd).

BP = blood pressure; BPV = blood pressure variability; LVMI = left ventricular mass index. A decrease or increase in LVMI was defined as changes in LVMI within the lower (a decrease greater than or equal to 1.7g/$m^{2.7}$) or upper (an increase greater than or equal to 1.7g/$m^{2.7}$) quartiles of the change in LVMI over 12 months.

Associations of BPV variables with ΔaPWV. There were no significant differences in the change in reading-to-reading or day-to-day BPV indices between the upper and the lower quartile of the aPWV changes with the exception of the night-time SBPV which was increased among participants who had an increase in aPWV (Table 3. 4). None of the changes in BPV indices, either reading-to-reading or day-to-day and neither visit-to-visit BPV indices were associated with ΔaPWV (Table 3. 3).

Changes in DDD and mean BP levels and BPV. Figure 3. 1 shows the comparison of the changes in mean BP levels and BPV between participants who had an increase and participants who had a decrease in DDD over time. As expected, for those participants who had an increase in DDD there was a corresponding decrease in all mean systolic and diastolic BP measures, whilst an increase in all mean BP levels measures was observed for those who had a decrease in DDD ($p<0.05$ for all). On the contrary, no significant differences were observed between groups with increased or decreased DDD in terms of BPV indices, with the exception of the day-to-day SBPV which was paradoxically increased for those who had an increase in DDD ($p=0.03$).

Table 3. 3 Multivariable associations between mean BP levels and BPV indices with the changes in aPWV and LVMI over time

Variable	**ΔLVMI (g/m$^{2.7}$) (n=267)**		**ΔaPWV (m/s) (n=250)**	
	Univariable	Multivariable§	Univariable	Multivariable§
Change in 24-hour ambulatory BP (mmHg)				
24-hour systolic BP	0.03 (0.01,0.06)†‖	0.03 (0.01,0.06)†	0.03 (0.01,0.05)†	0.03 (0.01,0.05)†
Daytime systolic BP	0.03 (0.01,0.05)*	0.03 (0.01,0.06)*	0.03 (0.01,0.05)†	0.03 (0.01,0.05)†
Night-time systolic BP	0.03 (0.01,0.05)†	0.03 (0.01,0.05)†	0.02 (0.01,0.03)*	0.02 (0.01,0.03)*
24-hour diastolic BP	0.04 (-0.01,0.09)	0.04 (-0.01,0.09)	0.04 (0.08,0.01)†	0.04 (0.01,0.08)†
Daytime diastolic BP	0.03 (-0.01,-0.08)	0.03 (-0.01,0.08)	0.04 (0.07,0.01)†	0.04 (0.01,0.07)†
Night-time diastolic BP	0.02 (-0.01,0.06)	0.02 (-0.01, 0.06)	0.02 (-0.03,0.05)	0.02 (-0.04,0.04)
Change in 7-day home BP (mmHg)				
Systolic BP	0.01(-0.02,0.04)	0.01 (-0.02,0.04)	0.01 (-0.01,0.03)	0.01 (-0.02,0.03)
Diastolic BP	0.00 (-0.05,0.05)	0.00 (-0.05,0.06)	0.02 (-0.01.0.06)	0.02 (-0.02.0.06)
Change in clinic BP (mmHg)				
Systolic BP	0.00 (-0.02,0.02)	0.00 (-0.02,0.02)	0.04 (0.02,0.05)‡	0.04 (0.02,0.05)‡
Diastolic BP	0.02 (-0.01,0.06)	0.02 (-0.01,0.02)	0.05 (0.02.0.07)‡	0.05 (0.02.0.07)‡
Change in reading–to-reading BPV (%)				
24-hour systolic BPV	-0.08 (-0.16,-0.01)*	-0.06 (-0.14,0.01)	0.01 (-0.04,0.06)	0.01 (-0.04,0.06)

Daytime systolic BPV	-0.06 (-0.13,0.01)	-0.06 (-0.13,0.01)	-0.01 (-0.05,0.05)	0.00 (-0.05,0.05)
Night-time systolic BPV	-0.01 (-0.05,0.05)	0.00 (-0.04,0.05)	0.03 (-0.03,0.06)	-0.03 (-0.04,0.07)
24-hour diastolic BPV	0.03 (-0.02,0.08)	0.02 (-0.02,0.07)	-0.01 (-0.04,0.02)	-0.01 (-0.04,0.02)
Daytime diastolic BPV	0.02 (-0.02,0.06)	0.02 (-0.02,0.07)	0.01 (-0.03,0.02)	0.01 (-0.03,0.02)
Night-time diastolic BPV	0.01 (-0.05,0.03)	0.01 (-0.05,0.03)	0.01 (-0.04,0.01)	0.01 (-0.04,0.01)
Change in day-to-day BPV (%)				
Systolic BPV	-0.01 (-0.15,0.13)	-0.01 (-0.15,0.13)	0.03 (-0.07,0.13)	0.04 (-0.06,0.14)
Diastolic BPV	-0.02 (-0.13,0.08)	-0.02 (-0.12,0.09)	0.00 (-0.06,0.08)	0.00 (0.01,0.16)
Visit-to-visit mean BP (mmHg)				
Clinic systolic BP	-0.02 (-0.04,0.01)	-0.03 (-0.06,0.01)	0.00 (-0.02,0.02)	0.00 (-0.07,0.08)
Clinic diastolic BP	0.00 (-0.03,0.04)	0.00 (-0.04,0.04)	0.00 (-0.03,0.03)	0.00 (-0.03,0.03)
Visit-to-visit BPV (%)				
Clinic systolic BPV	-0.05 (-0.15,0.06)	-0.03 (-0.14,0.08)	0.03 (-0.04,0.11)	0.03 (-0.05,0.11)
Clinic diastolic BPV (%)	-0.01 (-0.03,0.01)	-0.01 (-0.03,0.01)	0.06 (-0.07,0.02)	0.01 (-0.09,0.02)

*p<0.05, †p<0.01, ‡p<0.001.
[§]Adjusted for baseline age, sex, BMI.
[‖]Beta coefficient (95% confidence interval) (all such values).
ΔLVMI = change in left ventricular mass index; ΔaPWV = change in aortic pulse wave velocity; BP = blood pressure; BPV = blood pressure variability.

Table 3. 4 Changes in mean BP and BPV indices among participants with a decrease or increase in aPWV over 12 months.

Variable	Decrease in aPWV (n=63)	Increase in aPWV (n=62)	P value
24-hour ambulatory BP (mmHg)			
24-hour systolic BP	-1.50(11.94)	6.69(13.10)	0.001
Daytime systolic BP	-2.04(10.90)	7.06(12.68)	<0.001
Night-time systolic BP	0.15(16.36)	5.96(17.49)	0.08
24-hour diastolic BP	-0.41(7.50)	3.38(7.14)	0.009
Daytime diastolic BP	-0.76(6.30)	3.50(7.94)	0.003
Night-time diastolic BP	1.96(8.62)	3.45(8.91)	0.39
7-day home BP (mmHg)			
Systolic BP	1.87(10.83)	2.33(10.88)	0.82
Diastolic BP	-0.33(5.04)	2.70(5.65)	0.002
Clinic BP (mmHg)			
Systolic BP	-6.01(12.92)	5.15(14.23)	<0.001
Diastolic BP	-3.37(8.37)	2.39(9.23)	<0.001
Reading-to-reading BPV (%)			
24-hour systolic BP	-0.62(3.06)	-0.07(3.92)	0.42
Daytime systolic BPV	3.52(3.88)	4.23(4.56)	0.39
Night-time systolic BPV	-0.16(4.66)	1.08(5.54)	0.22
24-hour diastolic BPV	-1.52(6.06)	-0.12(5.90)	0.23
Daytime diastolic BPV	-0.45(6.21)	-0.14(6.00)	0.79
Night-time diastolic BPV	-1.33(5.98)	0.14(9.00)	0.33
Day-to-day BPV (%)			
Systolic BPV	-0.48(2.06)	-0.35(2.28)	0.74
Diastolic BPV	-0.71(2.65)	-0.11(2.83)	0.22

Data presented as mean(sd).
BP = blood pressure; BPV = blood pressure variability; aPWV= aortic pulse wave velocity. A decrease or increase in aPWV was defined as changes in aPWV within the lower (a decrease greater than or equal to 0.9m/s) or upper (an increase greater than or equal to 1m/s) quartiles of the change in aPWV over 12 months.

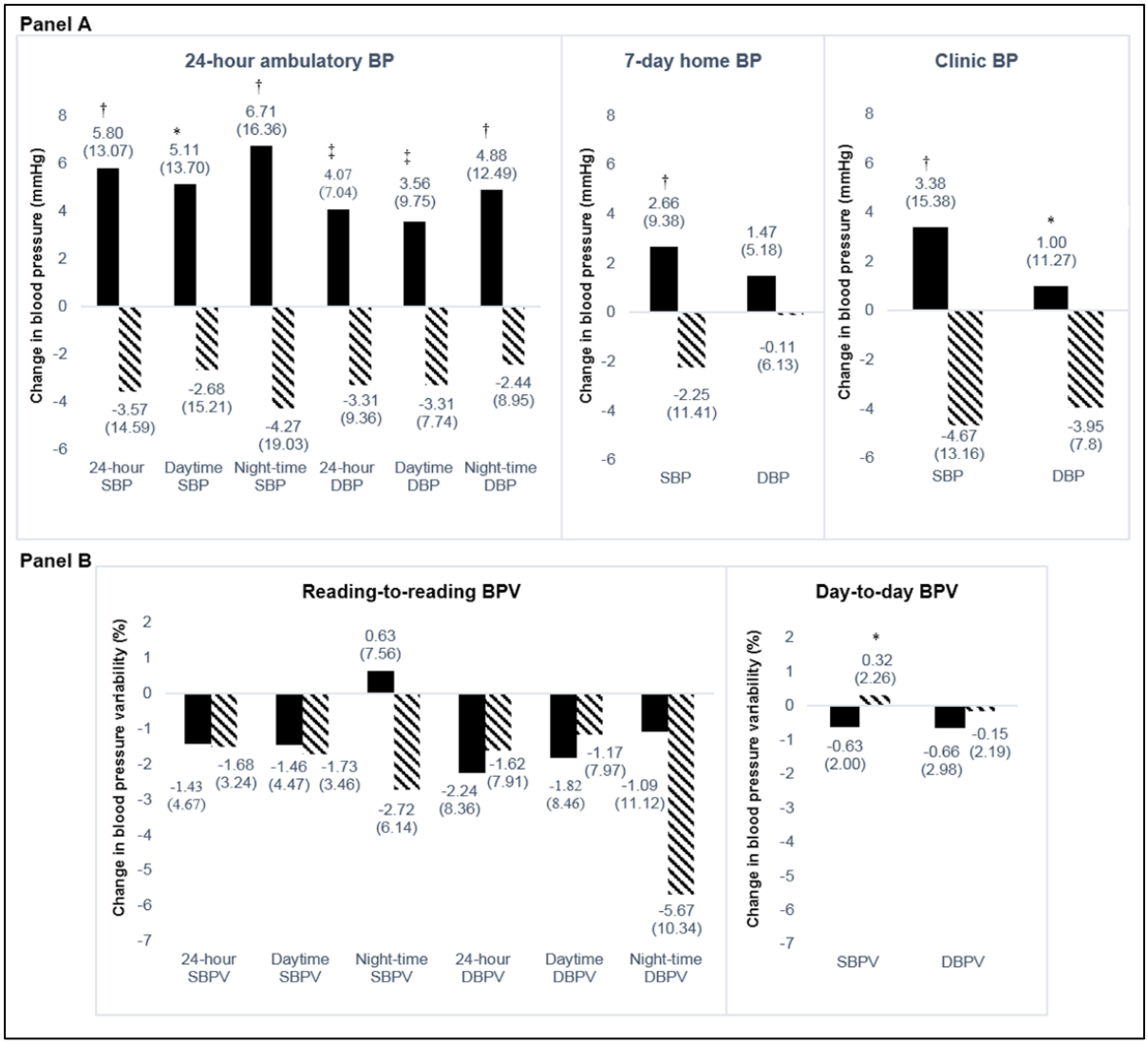

Figure 3. 1 Comparison of the changes in mean BP and BPV indices among participants who had a change in daily defined dose.
Solic colour represesnts participants who had a decrease in daily defined dose (n = 72) and pattern fill represents participants who had an increase (n = 40) over 12 months. (a) Changes in mean BP levels and (b) changes in BPV indices. *P < 0.05, †P < 0.01, ‡P < 0.001. BP; blood pressure; BPV, blood pressure variability.

Sensitivity analysis. Comparable results were observed for BPV indices calculated using the sandard deviation instead of the CV. Results were unchanged for the reading-to-reading BPV indices after exclusion of the periods from 20:00 to 22:00 and 06:00 to 08:00 and, for visit-to-visit BPV when analysis was repeated using the 7-day home BP instead of the clinic BP measures. Comparable results were also observed when day-to-day BPV was assessed separately for morning, midday or evening measurements.

3.5 Discussion

To our knowledge, this is the first reported follow-up study that has investigated the concept that the changes in TOD over time, as determined from cardiac structure and aortic stiffness, may not only depend on the changes in the magnitude of mean BP levels but also on the changes in the magnitude of BP fluctuations. Importantly, this is also the first study to investigate the effect on TOD from changes in BPV using clinic as well as out-of-office BP measures. The main findings were: i) none of the changes in BPV were associated with ΔLVMI or ΔaPWV independent of the mean BP levels and ii) only the changes in mean BP levels were related to the changes in TOD. Additionally, antihypertensive treatment titration had a clear and expected impact only on the changes in mean BP levels (i.e. decreased mean BP with increased DDD and vice versa) but with variable and sometimes paradoxical responses on BPV (Figure 3.1). These findings suggest that, at least from the point of view of predicting changes in TOD or effects of medications, BPV does not provide additional clinical information over and above mean BP levels in participants with uncomplicated hypertension.

Previous cross-sectional studies have also reported a lack of significant associations between reading-to-reading or day-to-day BPV measures and LVMI in hypertensive patients.[62-66] Interestingly, one of the most cited works on BPV, in which patients were followed for up to 7.4 years with BP monitored invasively at baseline, reported a positive association between ΔLVMI and baseline reading-to-reading BPV.[67] However, the sample size of that population was small (n=73) and was limited due to the lack of a follow-up assessment of BPV. A recent follow-up study also reported no association between LV hypertrophy (LVH) and long-term BPV; however, LVH was defined by electrocardiogram (a less sensitive method than echocardiography).[68] Other, cross-sectional studies [69-73] have found significant associations between reading-to-reading BPV and LVMI, independent of the mean BP levels. It is worth

noting though that these studies have used SD to quantify BPV which could have affected the results as even small degrees of multicollinearity, resulting from the well-known relationship between mean BP level and BPV, could affect the coefficients of the individual predictors in a model. Moreover, we are unaware of data available on visit-to-visit BPV with only one cross-sectional study reporting a lack of significant associations between day-to-day BPV and LVMI.[66] Lastly, although large artery stiffness has been suggested to affect BPV,[51,74] this is the first study that has investigated whether ΔaPWV were associated with changes in various BPV measures. Changes in BPV did not contribute in ΔaPWV, and our analyses clearly supports the dependence of aPWV on mean BP levels, as is well known.[53] These findings are in line with recent cross-sectional studies reporting no associations between reading-to-reading BPV[75] and day-to-day BPV,[66] although Wei et al. found a significant association between reading-to-reading BPV and aPWV in untreated hypertensive patients[66] and Webb et al. reported a significant association between day-to-day BPV and aPWV in patients with transient ischemic attack or minor stroke.[75] Additionally, Song et al. found that visit-to-visit BPV was associated with ΔaPWV in hypertensive patients, independent of the mean visit-to-visit BP levels,[76] but this study did not adjust for changes in mean BP levels over the follow-up period, something that could attenuate the association observed. Lastly, carotid intima-media thickness, a marker of atherosclerosis, was not associated with visit-to-visit BPV in mild-to-moderate hypertensive patients followed for 4 years.[26]

In vivo evidence suggests that an augmented BPV could cause a decrease in arterial distensibility and lead to aortic damage that could trigger LVH.[77] Similarly, it has been proposed that the mechanical stimuli resulting from an increased BPV could trigger the release of growth factors through the stimulation of mechanosensitive pathways that involve the local renin-angiotensin system and thus cause LVH.[78] Furthermore, increased BPV caused by an increased number of low BP episodes could deregulate cellular metabolism leading to

hypoperfusion and eventually LVH. Indeed, many authors have supported the notion that BPV may have some predictive value in humans in relation to TOD, but this could be modified by the level of cardiovascular risk.[26,39] In this current study population of patients with uncomplicated hypertension and a relative healthy vasculature in terms of aortic stiffness (baseline aPWV<10 m/s),[45] low prevalence of diabetes (<10%), and without conditions such as severe LVH or pre-existing coronary artery disease or renal disease, it seems that the susceptibility of the heart and large elastic arteries to BPV-related damage is low, at least within the timeframe of this study.

The strengths of this study include the follow-up design that allowed the assessment of the relationship between the changes in the outcomes and the changes in BPV measures, as well as the variety of BPV types that were assessed in the same population and the concurrent assessment of the change in DDD. On the other hand, the analysis relating to reading-to-reading BPV may be limited as the time intervals between readings were greater than 20 minutes and therefore longer than has previously been recommended.[2] However, cross-sectional studies that have used time-intervals less than 15 minutes have also reported no significant associations between BPV and LVMI.[62,66] Additionally, the results of the analysis on the change in DDD may be limited by the lack of a sub-analysis on medication class as different classes may have different effects on BPV.[79] However, an analysis regarding different antihypertensive medication classes was not possible due to the lack of statistical power. Moreover, day-to-day BPV was quantified using self-report BP monitoring which might be subject to bias. Lastly, the follow-up period might not have been adequate to investigate structural changes in LVMI and aPWV, and this may also help to explain the weak associations observed between the changes in mean BP levels and ΔLVMI.

This study provides evidence that changes in reading-to-reading, day-to-day BPV as well as visit-to-visit BPV do not contribute substantially to ΔLVMI or ΔaPWV and thus may not offer

any incremental prognostic value over and above mean BP levels in a population with uncomplicated hypertension. This is in agreement with previous suggestions that BPV may not have a predictive value in populations with low to moderate cardiovascular risk.[26,39] Additionally, this study shows that when accounting for the change in antihypertensive dose, BPV may be a phenomenon which, coupled with the need to keep mean BP levels controlled, may not be an easily treatable target by common antihypertensive treatment. In conclusion, the main message of this study is that it would seem reasonable for clinicians to remain focussed on mean BP levels when tailoring hypertension management decisions rather than BPV in patients not subject to cardiovascular risk factors beyond uncomplicated hypertension.

3.6 Contribution of chapter 3 to thesis aims

Chapter 3 (study 3) is the first longitudinal follow-up study which has examined the prognostic value of the main three types of BPV (short-, mid- and long-term) in the same population. Moreover, in contrast to the majority of studies which have investigated BPV in relation to cardiovascular risk in patients with high cardiovascular risk, the study sample comprised patients with well controlled hypertension and low to moderate cardiovascular risk. This is particularly important as the sample allows to specifically test the predictive value of BPV over and beyond that of mean BP levels. Study 3 showed that changes in mean BP levels, but not BPV, were most relevant to changes in organ damage in patients with uncomplicated hypertension. Therefore, BPV appears to offer limited clinical utility in this patient population.

Chapter 4

Age-dependent changes in blood pressure over consecutive office measurements: impact on hypertension diagnosis and implications for international guidelines

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4.1 Abstract

Objectives: Based on anecdotal belief that blood pressure (BP) drops over consecutive measurements, guidelines recommend discarding the first BP reading (Canadian Hypertension Education Program guidelines; CHEP), or take only one reading if systolic BP (SBP) <140 mmHg (National Institute for Health and Care Excellence; NICE). However, the extent to which SBP fluctuations affect BP classification as well as the potential effect of age, are unknown. We sought to assess the change in SBP classification over consecutive measurements following different guidelines, among younger (<50 years) and older individuals (≥50 years). Furthermore, we aimed to investigate the direction of the change in SBP over consecutive measurements (increase or decrease), and the impact of age on SBP differences.

Methods: BP was measured among 20,716 adults from a general population. SBP was classified using the first reading (normal SBP or hypertension) and compared with the average SBP using different guideline protocols (reclassification).

Results: Reclassification from normal SBP to hypertension was greatest with CHEP guidelines (3% younger, 12% older individuals) and reclassification from hypertension to normal SBP was greatest with NICE guidelines (70% younger, 44% older individuals). SBP increased between the first two measures in 37%, decreased in 56% and did not change in 7% of the population. Age had a strong interaction with SBP level ($p<0.0001$) so that younger individuals exhibited greater SBP differences over repeated measures.

Conclusions: This study highlights the need for an improvement in the evidence-base regarding the best way to assess office BP for correct hypertension diagnosis.

4.2 Introduction

Blood pressure (BP) measurement in the office remains the principal method for diagnosis and management of hypertension.[80] Office BP is a less reliable assessment of the true underlying BP in comparison to 24-hour ambulatory BP, which is considered the reference standard.[81] In the United States, an office BP assessment that overestimates or underestimates BP by as little as 5 mmHg could result in up to 21 million people being falsely diagnosed with hypertension, or up to 27 million people misdiagnosed as normotensive, thus leading to inappropriate hypertension management.[82,83] A common belief around office BP measurement is that the first BP reading overestimates the true BP as a result of a stress reflex response (alarm reaction) to the measurement procedure or the presence of the clinician. For this reason, the second and third BP measurements are thought to correspond more closely to the true BP. Importantly, these beliefs are not based on empirical data from large population samples.

The lack of empirical data has led to divergent recommendations among different international guidelines. Briefly, these recommendations include not taking a second BP if initial office BP is ≤140/90 mmHg and discarding the first measurement (Canadian Hypertension Education Program guidelines; CHEP),[84,85] or recording only the lower of the last two measurements (National Institute for Health and Care Excellence; NICE guidelines; Table 4. 1).[19] The European Societies of Hypertension and Cardiology (ESH/ESC) recommend taking two BP readings and only taking a third reading if "the first two are quite different" (>10 mmHg)[80] whilst the 7th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC–7), recommend taking at least two readings (Table 4. 1).[86] The 2016 Australian guidelines are in part in alignment with the CHEP guidelines recommending to take three BP readings and average the last two; however, if readings vary more than 10/6 mmHg for SBP/diastolic BP it is recommended to re-measure after 5 minutes of rest.[87]

Table 4. 1 Office blood pressure measurement protocols according to different international guidelines

BP level (mmHg)	CHEP *	NICE †	ESH/ESC ‡	JNC-7 §
If SBP1 <140	No further readings	No further readings		
If SBP1 ≥140	• Take a 2nd reading	• Take a 2nd reading		
If |ΔSBP| > 10	• Take a 3rd reading • Discard SBP1 • Consider the average of SBP2 and SBP3	• Take a 3rd reading • Discard SBP1 • Consider the lower of SBP2 and SBP3	• Take a 3rd reading • Consider the average of SBP1, SBP2 and SBP3	
				At least 2 readings

SBP1 denotes the first reading of systolic blood pressure (SBP); |ΔSBP| denotes the absolute difference between SBP1 and SBP2. *CHEP = Canadian Hypertension Education Programme. Hypertension. †NICE = National Institute for Health and Care Excellence.‡ESH/ESC = European Society of Hypertension/International Society of Hypertension. §JNC-7 = 7th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

Importantly, the accuracy of office BP in determining true BP control could be affected by spontaneous variability in BP over seconds or minutes.[88,89] Moreover, the difference in BP over consecutive measurements increases with increasing BP[90-92] and age,[91,92] and the diagnostic accuracy of office BP changes across the age span.[93,94] However, the extent to which the interaction between BP level and age could affect the magnitude or the direction (increase or decrease) of the change in BP over consecutive measurements and, consequently, the diagnosis of hypertension using office BP measures, is unknown. This study sought to determine the change in SBP classification over consecutive measurements based on guideline protocols (CHEP, NICE, ESH/ESC, JNC–7), among younger (<50 years) and older individuals (≥50 years). We also sought to determine the direction of the change in SBP over consecutive measurements as well as the interaction between SBP level and age on the differences in SBP. We hypothesised that SBP would not decrease systematically with consecutive readings, and that the SBP differences between readings would be dependent on age, thus affecting reclassification of diagnosis differently in younger and older individuals.

4.3 Methods

Survey population. Data analysed were taken from the Core Content and National Health Survey components of the Australian Health Survey (AHS),[95] conducted between 2011 and 2013 by the Australian Bureau of Statistics (ABS). The survey was a nationally representative sample of individuals from 20,500 private dwellings across Australia (n=31,837).[96] Data on 20,716 individuals (≥18 years old) who had two BP readings in the AHS were included in the primary analysis and secondary analysis was performed among participants who had three BP readings (n=5,189). An age threshold of 50 years was used to delineate younger from older individuals based on the well documented age-related changes in haemodynamic patterns after age 50.[97] Additional information on survey design, ethics approval, anthropometry, statistical details, BP exclusionary criteria, classification of BP control, anti-hypertensive medications,

biomedical measures and health conditions are provided in supplementary material (Appendix 2).

Blood pressure. Duplicate BP readings were taken by non-clinicians using a validated,[98] automated BP monitor (A&D:UA-851) on the left arm using an appropriate cuff size at the end of a survey interview with participants seated and relaxed. If there was a difference between the first and second BP reading of >10 mmHg in either SBP or DBP, then a third reading was taken.[99] BP measurements were consecutive; the precise time of each measurement was not recorded. BP readings were excluded if they were outside the extremes of physiological range and/or measurement error was suspected. These exclusion thresholds were: SBP >260 or <70 mm Hg, DBP >150 or <40 mm Hg, and pulse pressure (PP) >150 or <20 mm Hg. Participants with only one BP measure were excluded from this analysis. The above measurement protocol enabled assessment of BP classification using all the international guidelines previously mentioned. Primary outcomes and measures included the reclassification of BP category, the direction of change from first (SBP1) to second SBP (SBP2) readings and the absolute difference between the SBP1 and SBP2 readings (|ΔSBP|). Reclassification of BP category was defined as the change of a participants' BP status either from hypertension at SBP1 (≥140 mmHg) to normal average SBP (average SBP<140 mmHg) based on the above protocols, or from normal SBP1 (<140 mmHg) to hypertension (average SBP≥140 mmHg) based on the above protocols. Therefore, for the purpose of examining reclassification; when BP is classified based on one reading we use the term "normal SBP1" or "high SBP1". When BP is classified based on the average of more than one readings we use the term "normal average SBP" or "high average SBP". The cut-off of 140 mmHg was chosen to show normality of SBP based on the ESH/ESC guidelines; however, we acknowledge that other definitions may apply according to other guidelines (i.e. 130-139 mmHg might refer to prehypertension). Table 4. 1 summarizes the CHEP, NICE, ESH/ESC and JNC-7 hypertension guideline recommendations.

Hypertension definition and classification of blood pressure category. For the primary analysis, hypertension was defined as SBP ≥140 mmHg based on five protocols relevant to international guidelines and the goals of this study. The five protocols presented in Table 4.1 required either one, two or three BP readings for BP classification and were compared based on reclassification as described previously: 1) the average of SBP1 and SBP2 (not taking into account the magnitude of ΔSBP), 2) the average of SBP1 and SBP2 if ΔSBP≥10 mmHg, 3) the average of SBP1, SBP2 and third SBP (SBP3) readings if ΔSBP≥10 mmHg, 4) the average of SBP2 and SBP3, discarding SBP1 and; 5) the lower of the last two SBP readings if ΔSBP≥10 mmHg. Following hypertension classification based on SBP, classification based on DBP ≥90 mmHg was also undertaken. SBP was classified as; low (SBP <90 mmHg), normal (90-129 mmHg), high normal (130-139 mmHg), grade I (140-159 mmHg), grade II (160-179 mmHg) and grade III (≥180 mmHg) hypertension. Similarly, DBP was classified as; low (<60 mmHg), normal (60-84 mmHg), high normal (85-89 mmHg), grade I (90-99 mmHg), grade II (100-109 mmHg) and grade III (≥110 mmHg) hypertension.

Health conditions. Participants were asked whether they had been diagnosed with a medical or health condition and whether this condition was: 1) still-current and long-term, 2) still current but not long-term or 3) not current.[100] The specific classification of long-term health conditions reported by the participants was based on the International Classification of Diseases.[101]

Anti-hypertensive medications. 20,500 of the individuals participating in the Australian Health Survey had also provided detailed information regarding the use of medications (including antihypertensives) which was retrieved from the National Health Survey, 2011-2012 component of the survey. Information regarding the use of medications as collected by the National Health Survey has been previously published.[102]

Statistical analysis. Means (continuous data) and percentages (categorical data) are reported as summary measures, together with 95% confidence intervals because analyses were weighted using weights supplied by the ABS. The analysis utilised person-weights[96] provided by the ABS, which ensured that any disproportionate sampling of certain groups was taken into account. Replicate weights provided by the ABS were used to calculate standard errors and 95% confidence intervals using the Jackknife delete-1 method. Linear regression was used to estimate the relationship of |ΔSBP| with SBP1 and age as predictor variables, with the square of SBP1 included to capture non-linearities and product terms (age x SBP1, age x squared of SBP1) used to capture interactions between age and SBP1. Final models included covariates to adjust for sex and body mass index. Further analysis was performed to evaluate the effect of cardiovascular disease or antihypertensive medications on the main findings. Analysis was also performed on secondary outcomes (supplementary material, [Appendix 2]; the difference between SBP2 and SBP3; the overall variability in 3 SBP measures [coefficient of variation; SBP CV]; the difference between DBP readings; the difference between pulse pressure (PP) readings). Stata 10 was used for all analyses (StataCorp, College Station, Tx). A two-sided p value <0.05 was considered significant.

4.4 Results

Population characteristics. The mean age of sample was 45 years (95%CI 45,46; range: 18-85), 50% male. Blood and urine biomarkers were within normal range on average (Table 4. 2). The prevalence of cardiovascular diseases was low (Table 4. 2). The prevalence of measured high SBP differed across age groups and across hypertension guidelines with the greatest prevalence observed using the JNC-7 protocol (Figure 4. 1). The differences in prevalence of high SBP across JNC-7, ESH/ESC, CHEP and NICE guidelines were greatest in the oldest old

(≥80 years old; Figure 4. 1; 49% [95%CI 44,54], 48% [95%CI 44,53], 44% [95%CI 40,48] and 43% [95%CI 39,47]).

Table 4. 2 Population demographic and clinical characteristics

Variable	**N**	**Mean or % (95%CI *)**
Age (years)	20716	45 (45,46)
Male (%)	9829	50 (50,51)
Body mass index (kg/m^2)	19768	27 (27,28)
Systolic blood pressure (mmHg)	20716	124 (123.62,123.8)
Diastolic blood pressure (mmHg)	20716	77 (76.6,77.0)
Medical conditions (self-reported; still, current and long-term)		
Diabetes (%)†	1347	5.7 (5.3,6.1)
High cholesterol levels (%)	1641	7.1 (6.7,7.5)
Heart disease (%)‡	385	1.6 (1.4,1.8)
Stroke (%)	169	0.7 (0.5,0.8)
Low blood pressure (%)	170	0.8 (0.7,0.9)
Cardiovascular disease biomarkers		
Total cholesterol (mmol/L)	8932	5.07 (5.04,5.10)
High density lipoprotein cholesterol (mmol/L)	8932	1.34 (1.33,1.35)
Fasting low density lipoprotein cholesterol (mmol/L)	7078	3.13 (3.10,3.15)
Fasting triglycerides (mmol/L)	7160	1.28 (1.26,1.31)
Apolipoprotein B (g/L)	8930	0.96 (0.95,0.97)
Diabetes biomarkers		
Fasting plasma glucose (mmol/L)	7161	5.10 (5.07,5.13)
Glycated hemoglobin (mmol/mol)	8915	36.13 (35.98,36.29)
Kidney disease biomarkers		
Albumin creatinine ratio (mg/mmol)	8467	1.81 (1.56,2.06)
Estimated glomerular filtration (mL/min/1.73m^2)	8927	85.62 (85.38,85.86)

Data are presented as mean or % (95%CI) *CI = confidence interval. Proportions and means are weighted to provide population estimates. †Includes type I, type II or high blood/urine sugar levels. ‡Includes ischaemic disease, heart failure and other heart disease.

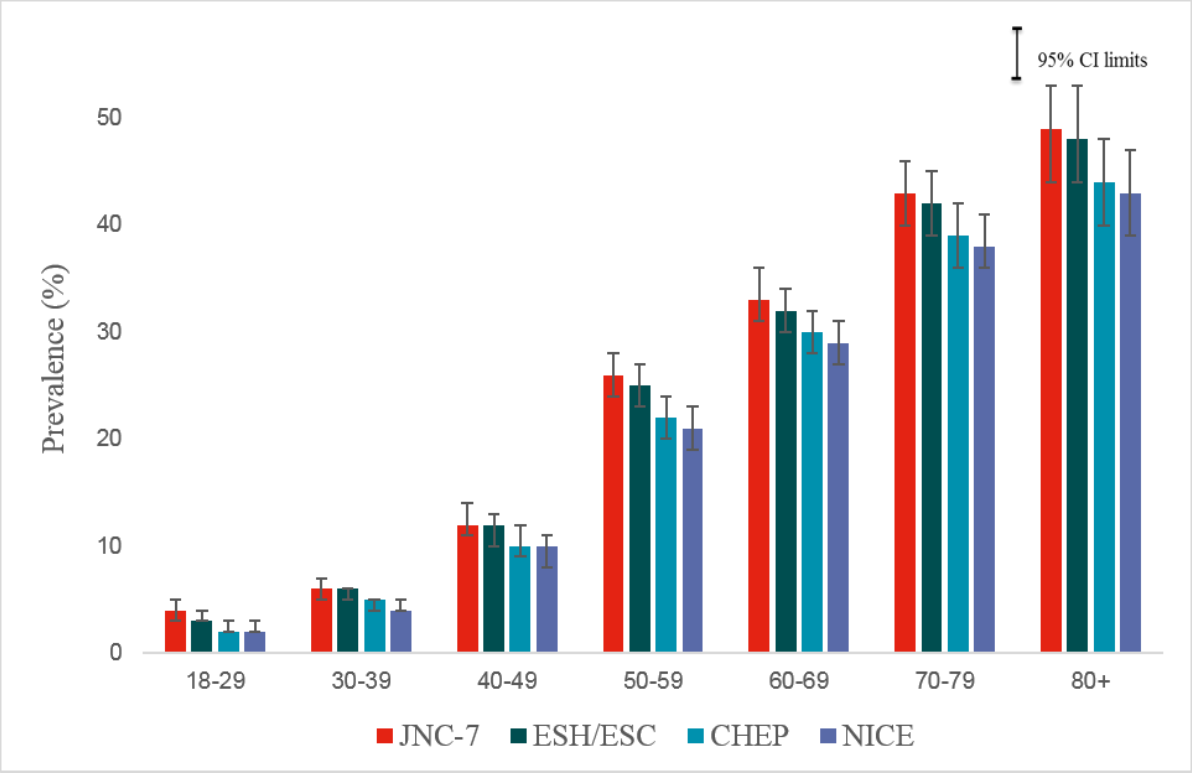

Figure 4. 1 Prevalence of high systolic blood pressure across age groups and different international protocols.
CHEP = Canadian Hypertension Education Programme. Hypertension. NICE = National Institute for Health and Care Excellence. ESH/ESC = European Society of Hypertension/International Society of Hypertension. JNC-7 = 7th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.

Reclassification of BP from normal SBP1 to high SBP. This reclassification was consistently greater for older individuals across all guideline protocols (Table 4. 3). When only two BP measures were used, 3% (95%CI 2,3) and 1% (95%CI 1,2) of older individuals with normal SBP1 were reclassified to high SBP according to JNC-7 and ESH/ESC guidelines, whilst such reclassification was observed in only 0.7% (95%CI 0.5,0.9) and 0.4% (95%CI 0.03,0.6) of younger individuals. The reclassification was greater when all three BP readings were used, and ranged from 7% to 12% for older individuals and 1% to 3% for younger individuals. The greatest reclassification from normal SBP1 to high SBP was observed using the CHEP guidelines (3% [95%CI 2,4] in younger versus 12% [95%CI 5,10] in older individuals).

Table 4. 3 Prevalence of hypertension and percentages of the population re-classified by use of each guideline protocol relative to classification based on a single systolic blood pressure reading as values of at least 140 mmHg

	Prevalence of hypertension*		**Re-classification by guideline protocol ***			
Guideline † (number of subjects with required data)	SBP1≥140 mmHg		From high SBP1 to normal average SBP		From normal SBP1 to high average SBP	
	Age<50yrs	Age≥50yrs	Age<50yrs	Age≥50yrs	Age<50yrs	Age≥50yrs
JNC-7 †	9%	35%	25%	10%	0.7%	3%
(n=20,716)	(8%, 9%)	(33%, 36%)	(21%, 29%)	(9%, 11%)	(0.5%,0.9%)	(2%, 3%)
ESH/ESC ‡	6%	31%	14%	5%	0.4%	1%
(n=15,527)	(6%, 8%)	(30%, 32%)	(11%, 18%)	(4%, 7%)	(0.3%,0.6%)	(1%, 2%)
ESH/ESC §	16%	46%	51%	25%	1%	7%
(n=5,189)	(14%, 18%)	(44%, 49%)	(45%, 57%)	(22%, 29%)	(1%, 2%)	(5%, 10%)
CHEP ‖	16%	46%	64%	35%	3%	12%
(n=5,189)	(14%, 18%)	(44%, 49%)	(57%, 71%)	(31%, 39%)	(2%, 4%)	(9%, 14%)
NICE ¶	16%	46%	70%	44%	2%	7%
(n=5,189)	(14%, 18%)	(44%, 49%)	(63%, 76%)	(41%, 47%)	(1%, 2%)	(5%, 9%)

*Data are presented as % (95%CI). SBP1 denotes the first reading of systolic blood pressure and SBP denote the average systolic blood pressure according to each guideline. †JNC-7 = 7th report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension defined as (SBP1+SBP2)/2 ≥ 140 mmHg. ‡ESH/ESC = European Society of Hypertension/International Society of Hypertension. Hypertension defined as (SBP1+SBP2)/2 ≥ 140 mmHg. §ESH/ESC. Hypertension defined as (SBP1+SBP2+SBP3)/3 ≥ 140 mmHg. ‖CHEP = Canadian Hypertension Education Programme. Hypertension defined as (SBP2+SBP3)/2 ≥ 140 mmHg. ¶NICE = National Institute for Health and Care Excellence. Hypertension defined as min(SBP2, SBP3) ≥ 140.

Reclassification of BP from high SBP1 to normal SBP. In contrast to the reclassification from normal SBP1 to high SBP, reclassification from high SBP1 to normal SBP occurred more frequently across all guideline protocols in younger individuals (range 14% to 70%) than in older individuals (range 5% to 44%) (Table 4. 3). The greatest reclassification was observed using the NICE guidelines (70% [95%CI 63,76] in younger versus 44% [95%CI 41,47] in older individuals). Differences among guideline protocols were observed when all three SBP readings were used (Table 4. 3).

Overall reclassification of BP. The overall reclassification from SBP1 (either, from normal SBP1 to high SBP or from high SBP1 to normal SBP) ranged from 1% to 12% for younger individuals and 3% to 24% for older individuals across guideline protocols. As expected overall reclassification was low when only SBP1 and SBP2 were used; for example 3% (95%CI 2,3) and 1% (95%CI 1,2) for younger individuals according to JNC-7 and ESH/ESC and 5% (95%CI 5,6) and 3% (95%CI 2,3), respectively, for older individuals. However, the differences in reclassification across guidelines became greater when all three SBP readings were used. The overall reclassification for younger individuals was 9% (95% CI 8,11), 12% (95%CI 10,14) and 12% (95%CI 11,14), according to ESH/ESC, CHEP and NICE guidelines, respectively. The overall reclassification for older individuals was 16% (95% CI 14,18), 21% (95%CI 19,23) and 24% (95%CI 22,26), according to ESH/ESC, CHEP and NICE guidelines, respectively.

Prevalence of increase versus decrease and no change from SBP1 to SBP2. Thirty-seven percent (95%CI 36,38) of the population had an increase, 56% (95%CI 55,57) had a decrease and 7% (95%CI 7,8) had no change in SBP from SBP1 to SBP2. When a tolerance of 5 mmHg was allowed so that an increase or decrease was defined as an SBP change of at least ≥5 mmHg in the respective direction, 18% (95%CI 18,19) had an increase, 33% (95%CI 32,33) had a decrease and 49% (95%CI 48,50) had no change in SBP (|ΔSBP| -4 to 4 mmHg). The

proportion of the population with an increase in SBP from SBP1 to SBP2 became lower with greater SBP1 (Figure 1, panel A; supplementary material [Appendix 2]). However, this proportion became higher with increasing age (Figure 1, panel B; supplementary material [Appendix 2]). The age-dependent direction for the change in SBP (increase or decrease) was similar when ΔSBP was redefined as a change of at least 5 mmHg in the respective direction (Figure 2; supplementary material [Appendix 2]).

BP variability indices. The overall mean ΔSBP was -1.67 (95%CI -1.81,-1.53) and the overall mean change irrespective of direction (absolute value) was 4.86 mmHg (95%CI 4.77,4.95). The difference observed between the overall magnitude of ΔSBP and |ΔSBP| was due to a high prevalence of an increase from SBP1 to SBP2 among the population; ΔSBP was smaller than the |ΔSBP| and did not accurately capture the magnitude of the change from SBP1 to SBP2. The overall SBP CV among three SBP readings was 6.56 mmHg (95% CI 6.42,6.70).

Interaction between the level of SBP1 reading and age. There was a statistically significant interaction between age and SBP1 level ($p<0.0001$) that resulted in younger individuals with higher SBP1 having greater changes from SBP1 to SBP2 (Figure 4. 2) than older individuals with comparable SBP1. As examples, the estimated |ΔSBP| for a person with SBP1 of 190 mmHg was 19.80 mmHg (95% CI 15.73,23.86) at age 35 years but 7.07 mmHg (95%CI 6.86,8.06) at age 75 years (Figure 4. 2; panel A). Similarly, when the overall direction of change is taken into account, the estimated ΔSBP for a person with SBP1 of 190 mmHg was -16.93 mmHg (95% CI -21.44,-12.44) at age 35 years but -5.52 mmHg (95%CI -6.97,-4.07) at age 75 years (Figure 4. 2; panel B).

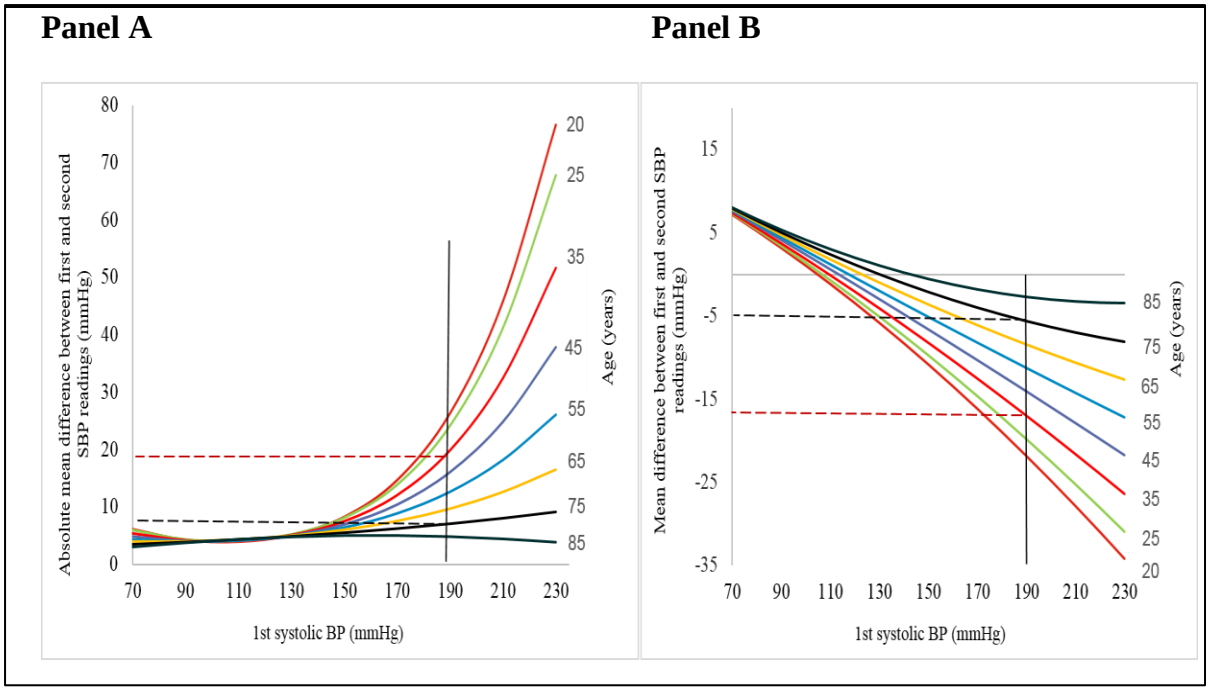

Figure 4. 2 Modifying effect of age on the relationship of the absolute difference (Panel A) or difference (Panel B) between first and second systolic blood pressure (SBP) readings and the level of the first SBP reading.
The corresponding absolute difference between the first and second SBP reading when the first SBP is 190 mmHg decreases from 20 mmHg for a 35 year old (red dotted line; panel A) to 7 mmHg for a 75 year old individual (black dotted line; panel A). The corresponding difference between the first and second SBP reading when the first SBP is 190 mmHg is -17 mmHg for a 35 year old (red dotted line; panel A) and -6 mmHg for a 75 year old individual (black dotted line; panel A).

Additional analysis. Results were unchanged when analysis was confined to the differences between SBP2 and SBP3 or when analysis took into account the total variation within three consecutive measurements (Figure 3; supplementary material [Appendix 2]). Results were also unchanged after adjustment for cardiovascular diseases. The interaction between age and SBP1 was further assessed in a sub-group of individuals who had information on anti-hypertensive medications; findings remained unchanged after adjusting for different classes of anti-hypertensive medications including antiarrhythmics, beta blockers, calcium channel blockers, diuretics, angiotensin receptor blockers, angiotensin converting enzyme blockers or other antihypertensive classes. ΔSBP was not significantly different across different anti-hypertensive medication classes (p=0.10) and results were similar when the difference was adjusted for mean SBP1 level and age (p=0.14). Analyses were also conducted using the DBP

or PP instead of the SBP readings; however, as compared to the SBP, there were no age-related patterns, nor were similar relationships observed in relation to the DBP difference between measurements. (supplementary material [Appendix 2]).

4.5 Discussion

Accurate measurement of office BP is critical in the diagnosis and management of hypertension. The assumption that an alarm reaction to BP measurement could affect the accuracy of office BP has led to uncertainty regarding the number of readings required for diagnosis. We observed substantial differences in reclassification across NICE, CHEP, ESH/ESC and JNC-7 guideline BP measurement protocols. In contrast to the assumption of a consistent alarm reaction resulting in BP dropping with repeat measurement, we found that SBP actually increased from SBP1 to SBP2 in more than a third, and did not change in 7% of the population. When we broadened the tolerance to define SBP change to be ≥5 mmHg between SBP1 and SBP2, 18% of the population still had an increase in SBP, whereas a third of the population had a decrease in SBP. Notably, there was a strong age modification effect on the relationship between ΔSBP and SBP1, indicating that the difference in SBP with repeat measurements decreased with increasing age. Altogether these data are highly relevant to international guideline recommendations on BP measurement for further evaluation or initiation of hypertension treatment.[80,84,19,85,86]

The NICE and CHEP hypertension guidelines recommend taking only one reading if SBP1 is <140 mmHg. However, based on our results (Table 4. 3), 7% and 12% of the older participants would have been misclassified as normotensive at SBP1 using NICE or CHEP guidelines respectively, whereas hypertension would have been diagnosed if a third reading had been taken. If we take the example of a 60-year-old individual with only one BP reading recorded as 138 mmHg then, based on either NICE or CHEP guidelines, they would be classified as

normotensive without any further assessments. However, as approximately 40% of older individuals have an increase from SBP1 to SBP2 (Figure 1, panel B; supplemental material [Appendix 2]), there is a reasonable possibility that such an individual would have SBP greater than 140 on repeat measurement and thus reclassified as hypertensive. While keeping in mind the value of management based on absolute cardiovascular risk assessment, older individuals have a 3-4 fold increase in cardiovascular disease risk,[86] and it is important to avoid missing a diagnosis of hypertension. On the other hand, our data suggest that younger individuals have greater propensity towards false positive hypertension diagnosis and potentially having unnecessary treatment initiated. Although all guideline BP measurement protocols that used three BP readings resulted in sizeable reclassification from high SBP1 to normal SBP among younger individuals, there were significant differences among ESH/ESC, CHEP and NICE BP measurement protocols. Indeed, using three BP readings, 50% of younger individuals with high SBP1 would reclassify to having normal SBP based on the ESH/ESC guidelines, 64% based on CHEP and 70% based on NICE guidelines.

Since office BP methods involving only a few BP readings over several minutes have been used for global estimates of hypertension prevalence, as well as in most BP-related epidemiological studies, it is difficult to directly compare BP statistics across different guideline protocols, and especially across age ranges. To our knowledge there is no evidence to support the diagnostic accuracy of any international hypertension protocols compared with the gold standard technique of 24 hour ambulatory BP or other out-of-office BPs such as home BP. Our data show that limiting BP assessment to only one BP reading is a flawed approach for assessing BP control and also support the argument that out-of-office BP methods should become part of routine BP assessment.[17,103,104] Automated unobserved office BP[105,106] measures may also be an appropriate alternative to more accurately measure and determine risk related to BP.

Short-term changes in BP under resting conditions result from a combination of homeostatic mechanisms.[89,107] Indeed, it is expected for persons with a lower-than-average first BP measurement to have a higher second measurement on average, and for those with a higher-than-average first BP to have a lower second reading on average (see Figure 1, panel A, supplementary material [Appendix 2]), indicating a biologically plausible regression towards the mean. Nonetheless, the impact of ageing on BP regulation is complex. It has been suggested that short-term BP regulation is under increased influence of sympathetic nerve activity (SNA) in older individuals, which could result in increased short-term BP variability.[108] However, smaller resting SBP changes in response to increased SNA have been shown in older individuals at rest,[109] in response to head-up tilt test[110] or to the Valsalva manoeuvre.[111] The attenuation in the level of BP changes observed in older individuals at rest is suggested[109,112] to be the result of a decreased effectiveness of SNA, due to either decreased α-adrenergic sensitivity or reduced norepinephrine release triggered by SNA associated with healthy ageing. On the basis of this we speculate that the smaller SBP differences observed with advanced age in this study may reflect a lower minute-to-minute BP regulatory ability in older individuals.

Strengths of our study include the large sample of nationally representative data from a population-based survey, as well as the BP measurement procedure which reflects the recommended way to perform office BP. Secondly, BP was assessed by non-clinicians and this may have lessened the "white coat response" triggered by the presence of a clinician.[113,114] On the other hand, the above may also be a limitation as a different ΔSBP response may have occurred if measurement was made by clinicians. Furthermore, it could be argued that the results may have been affected by using oscillometry as compared to auscultation; however; oscillometry takes out the effect of digit preference and operator's bias, thus may have strengthened the results. Moreover, although it is recommended for office BP measures to use the arm with the highest BP readings, the left arm was used for all participants as it was not

feasible to test BP in both arms during such a time-intensive large population study. However, large inter-arm differences are more likely to affect individuals with arterial diseases[115] and given that the results were unchanged after correcting for diseases of arteries, arterioles, and capillaries, we expect that this is not a major study limitation. Additionally, multiple office visits or out-of-office BP measures were not available to help confirm hypertension diagnosis, but results are still relevant to an office visit using methods similar to that employed in this study. Lastly, secondary analysis using all three BP readings has been performed on an incomplete data set because a third reading was only taken if there was ≥10 mmHg difference between the first and second BP readings, and was not a predefined methodological protocol. Thus, the results of analyses which included all three BP measures could be more relevant to populations with increased BP variability.

In conclusion, the assessment of BP is subject to marked and unpredictable variability leading to increased measurement and diagnostic uncertainty. Notably, the assumption that BP drops with consecutive measurements is incorrect. Furthermore, age significantly affects the difference in SBP observed in office BP measures with potential consequences for correct hypertension diagnosis. Altogether these findings underscore the importance of BP measurement guidelines that are evidence-based in order to avoid assumptions leading to diagnostic error. Lastly, our study highlights the need to use out-of-office BP measures (i.e. home or 24-hour BP) to confirm hypertension diagnosis.

4.1 Contribution of chapter 4 to thesis aims

Chapter 4 (study 4) represents the first study to evaluate the impact of within-visit BP fluctuations with repeat measurements on hypertension classification by comparing clinic BP protocols as defined by four international hypertension guidelines. This study indicated that BP does not necessarily decrease with repeat measurement and that both age and the level of

BP significantly affect the accuracy to correctly classify hypertension status. The need for an improvement in the evidence-base regarding the best way to assess office BP for correct hypertension diagnosis was underscored by the findings of this study.

Chapter 5

Influence of blood pressure level and age on within-visit blood pressure variability in children and adolescents

At the time of thesis submission, this chapter is under peer-review with Pediatric Research

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5.1 Abstract

Objective. Blood pressure (BP) is variable in children and this could affect BP assessment, but the magnitude of within-visit BP variability (BPV) over consecutive measurements has never been investigated. This study aimed to determine the direction and magnitude of, and factors affecting, within-visit BPV in children and adolescents.

Study design and setting. BP was recorded among 3047 children (aged 12 years [95%CI; 12,13], males 52%) from the 2011-2013 Australian Health Survey. BPV was defined as the absolute difference (ΔSBP_{ABS}) between the first (SBP1) and second systolic BP (SBP2), and the overall variability in three measures (SBPV).

Results. On average, ΔSBP_{ABS} was 6.7 mmHg (95%CI 6.3,7.0) and SBPV was 8.2 % (95%CI 10.0,11.2). ΔSBP_{ABS} was greater with higher BP levels but lower with older age. From first to second measurements, SBP decreased in 58% (95%CI 56,60); did not change in 10% (95%CI 9,12), and increased in 32% (95%CI 29,34) of the population.

Conclusions. BP is highly variable in children and adolescents, with the magnitude of variability being associated with both age and BP level. SBP increases on repeat measurement in a substantial proportion of the population. The optimal protocol of BP assessment to address this increased BPV needs to be determined.

5.2 Introduction

Elevated blood pressure (BP) in childhood and adolescence predicts development of future hypertension[116,117] and is associated with surrogate markers of cardiovascular disease risk, such as increased large artery stiffness,[118] left-ventricular hypertrophy,[119,120] increased carotid intima medial thickness,[121] atherosclerotic lesions[122,123] and retinal arteriolar narrowing.[124] However, accurate BP assessment in children and adolescents may be influenced by within-visit BP variability (BPV).[125,126] To date, there are no data outlining the magnitude of BPV, nor the factors affecting BPV among young people. Furthermore, it is also believed that accurate assessment of BP in childhood is affected by an increased rate of false-positive readings due to the normalization of BP with consecutive measurements.[127] Indeed, it has been suggested that BP systematically decreases with repeat measures within a visit, due to an 'accommodation effect,[128,129] whereby individuals become familiar with the BP measurement process and therefore better accommodate the stress related to this process. Also, current hypertension guidelines recommend repeated BP measurements,[130] but the number of measurements is not specified.

A lack of epidemiological studies with hard clinical end-points (cardiovascular events and mortality) among children and adolescents has led to the development of BP assessment criteria that rely on age, sex and height probabilistic criteria (children with BP values greater than a specific percentile are classified as having high BP). According to the Fourth Report of the National High Blood Pressure Education Program, pre-hypertension in youth is defined if BP is $\geq 90^{th}$ or $\geq$ 120/80 mmHg and hypertension is defined if BP is $\geq 95^{th}$ percentile of age, sex and height dependent BP criteria.[130] Importantly, the difference between the 90^{th} and 95^{th} percentiles is only 3-4 mmHg, thus the magnitude of within-visit BPV could substantially affect the diagnosis of hypertension. Previous work from our group[131] showed that within-visit BPV in adults was highly influenced by age and BP level and we hypothesized that the same

effect would be evident among in youth. Therefore, this study aimed to investigate the direction and magnitude of within-visit BPV indices as well as factors (e.g. age and BP level) affecting BPV indices among children and adolescents.

5.3 Participants and methods

Data on 3047 children and adolescents, aged 5 to 17 years old, from a nationally representative sample of 31 837 individuals from the Australian Health Survey 2011-2013[132] who had two or three BP readings were analyzed. Detailed information on survey design, data management and data accessibility was previously published[131] and is summarized in the supplementary material (Appendix 3).

Anthropometry. Body mass index (BMI; kg/m^2) was calculated as weight (kg) divided by $height^2$ (m^2). For validation purposes, 10% of the participants were randomly selected for a second height measurement and if there was a difference greater than one centimetre then a third reading was taken.[133] Waist circumference (cm) was also measured, according to guidelines.[134] Height and BMI percentiles for age and sex were defined according to the United States Centers for Disease Control clinical growth charts.[135] Overweight status was defined based on a BMI at, or above the 85^{th} BMI percentile for age and sex as recommended.[135]

Blood pressure. Duplicate BP readings were taken by non-clinicians using an automated BP monitor (A&D:UA-851)[98] on the left arm and using an appropriate cuff size at the end of a survey interview with participants seated and relaxed. If there was a difference between the first and second BP reading of >10 mmHg in either SBP or DBP, then a third reading was taken (n=929).[99] Hypertension category was based on the average of the first two BP readings because a third reading was not available for all participants. Hypertension was defined if the average of the first two SBP or DBP readings were $\geq 95^{th}$ percentile of BP reference values for

age, sex and height.[136] Normal BP included pre-hypertension levels (≥90th percentile of BP reference values).

Blood pressure variability (BPV). The primary measures were defined as the difference (ΔSBP) between the first (SBP1) and second SBP (SBP2) readings, or the absolute difference (ΔSBP_{ABS}) between SBP1 and SBP2, which did not take into account the direction of the change, and the overall variability in three BP measurements calculated as the coefficient of variation (SBPV; [standard deviation/mean SBP]x100).

Statistical analysis. Linear regression was used to test for between-groups differences. Linear regression was used to estimate the relationship of ΔSBP and ΔSBP_{ABS} with SBP1 and age as predictor variables. The square of SBP1 was included to capture non-linearity and, product terms (age x SBP1, age x square of SBP1) were used to capture interactions between age and SBP1. Final models included covariates to adjust for height, sex and body mass index. Differences in the product terms (age x SBP1, age x square of SBP1) among female and male participants were assessed by including sex and three-way interaction terms among age, sex and the level of SBP1 (age x sex x SBP1, age x sex x squared SBP1) in a fully saturated model. Analyses was repeated on the overall variability in 3 SBP measures (SBPV) or the difference between DBP readings. Stata 10 was used for all analyses (StataCorp, College Station, Tx).

5.4 Results

The population was on average 12 years old (95%CI 12,13) and 52% male. The prevalence of hypertension was 6% (95%CI 5,7). Demographic and clinical characteristics of the population are presented in Table 5. 1. Mean SBP and DBP increased with age ($p<0.001$); however, SBPV and DBPV decreased with age ($p<0.001$; Table 5. 1).

Table 5. 1 Demographic and clinical characteristics of the population (n=3047).

Variable	5-7 yrs (n=261)	8-10 yrs (n=510)	11-13 yrs (n=864)	14-17 yrs (n=1414)	Between-group p value
Age (years)	6.1 (6.0,6.3)	9.1 (9.0,9.2)	12.0 (11.9,12.1)	15.6 (15.5,15.7)	<0.0001
Male (%)	57 (49,64)	52 (47,56)	52 (59,55)	52 (49,54)	0.64
BMI (kg/m^2)	17.7 (17.1,18.4)	18.9 (18.5,19.2)	20.5 (20.2,20.8)	22.4 (22.1,22.7)	<0.0001
Overweight (%)	41 (32,49)	37 (31,43)1	32 (28,35)	26 (23,30)	0.015
*Hypertension (%)	8 (3,12)	7 (4,9)	5 (3,7)	6 (4,8)	0.40
†SBP (mm Hg)	96.9 (94.2,99.6)	100.4 (98.9,101,8)	105.5 (104.5,106.6)	110.9 (110.0,111.8)	<0.0001
†DBP (mm Hg)	60.6 (58.4,62.8)	63.4 (62.3,64.5)	65.7 (64.9,66.6)	67.0 (66.3,67.7)	<0.0001
ΔSBP_{ABS} (mm Hg)	7.07 (5.1,9.0)	7.2 (6.2,8.2)	6.5 (6.1,7.0)	6.5 (6.0,6.9)	0.56
ΔDBP_{ABS} (mm Hg)	6.01 (4.7,7.3)	5.7 (5.0,6.3)	5.1 (4.6,5.6)	5.3 (5.0,5.7)	0.34
‡SBPV (%)	11.8 (8.7,14.8)	9.1 (8.0,10.2)	8.2 (7.4,9.04)	7.0 (6.6,6.9)	<0.001
‡DBPV (%)	16.5 (13.0,20.0)	11.3 (10.0,13.0)	9.7 (9.0,11.0)	9.7 (9.0,11.0)	<0.001

Data are presented as mean (95% CI) or % (95%CI). BP denotes blood pressure; SBP denotes systolic BP; DBP denotes diastolic BP; ΔSBPABS denotes the absolute difference between the first and second SBP readings; ΔDBPABS denotes the absolute difference between the first and second DBP readings; SBPV denotes systolic BP variability; DBPV denotes diastolic BP variability. *According to age-, sex- and height-dependent blood pressures thresholds. †Based on the average of two blood pressure readings. ‡Estimates are based on a subpopulation with three blood pressure readings (n=929).

On average, ΔSBP was -2.4 mmHg (95%CI -2.0,1.7) and ΔSBP_{ABS} was 6.7 mmHg (95%CI 6.3,7.0). For the population with three BP readings, the overall SBPV was 8.2 (95%CI 10.0,11.2). The magnitude of SBPV and ΔSBP_{ABS} were greater among children with hypertension compared to children with normal BP (SBPV; 10.5 % [95%CI; 8.4,12.3] vs 7.8 % [95%CI; 7.5,8.3], p=0.005 and ΔSBP_{ABS}; 11.7 mmHg [95%CI; 8.4,15.0] vs 6.4 mmHg [95%CI; 6.0,6.7]; p=0.002). Additionally, there was some evidence that the magnitude of

SBPV and ΔSBP_{ABS} were greater among males compared with females (SBPV; 8.6 % [95%CI; 7.9,9.3] vs 7.7 % [95%CI; 7.1,8.2], p=0.04 and ΔSBP_{ABS}; 7.1 mmHg [95%CI; 6.6,7.6] vs 6.2 mmHg [95%CI; 5.7,6.7]; p=0.01).

A non-linear association was observed between ΔSBP_{ABS} or ΔSBP and the first SBP reading which was modified by age for both female and male participants ($p<0.0001$ and p=0.0002 respectively; Figure 5. 1). The interaction between age and the level of the first SBP reading indicated a smaller difference in SBP for adolescents compared with younger children for any given level of first SBP reading, with differences across age being more pronounced among female participants (Figure 5. 1; p=0.037). A similar interaction was observed between mean SBP level and the total SBPV in the subpopulation with 3 BP readings, resulting in a modification effect of age on the relationship between mean SBP level and overall SBPV over 3 readings (p=0.03 for male and p=0.004 for female participants). When SBP was replaced by PP, the same interaction was observed between the level of initial PP and the difference between the first and second PP readings (interaction $p<0.001$ and p=0.026, for males and females, respectively). No age-dependent relationships were observed for either the DBP difference between measurements or the overall variability in three DBP readings when analysis was performed using DBP instead of SBP.

From the first to second measurement, SBP decreased in 58% (95%CI 56,60); did not change in 10% (95%CI 9,12), and increased in 32% (95%CI 29,34) of the population. Allowing for a tolerance in the difference between the first to second SBP measurements of 5 mmHg or more, still resulted in 34% (95%CI 33,37) experiencing a decrease in SBP, whereas 16% (95%CI 14,17) had an increase and 50% (95%CI 48,52) had no change in SBP (ΔSBP -4 to 4 mmHg).

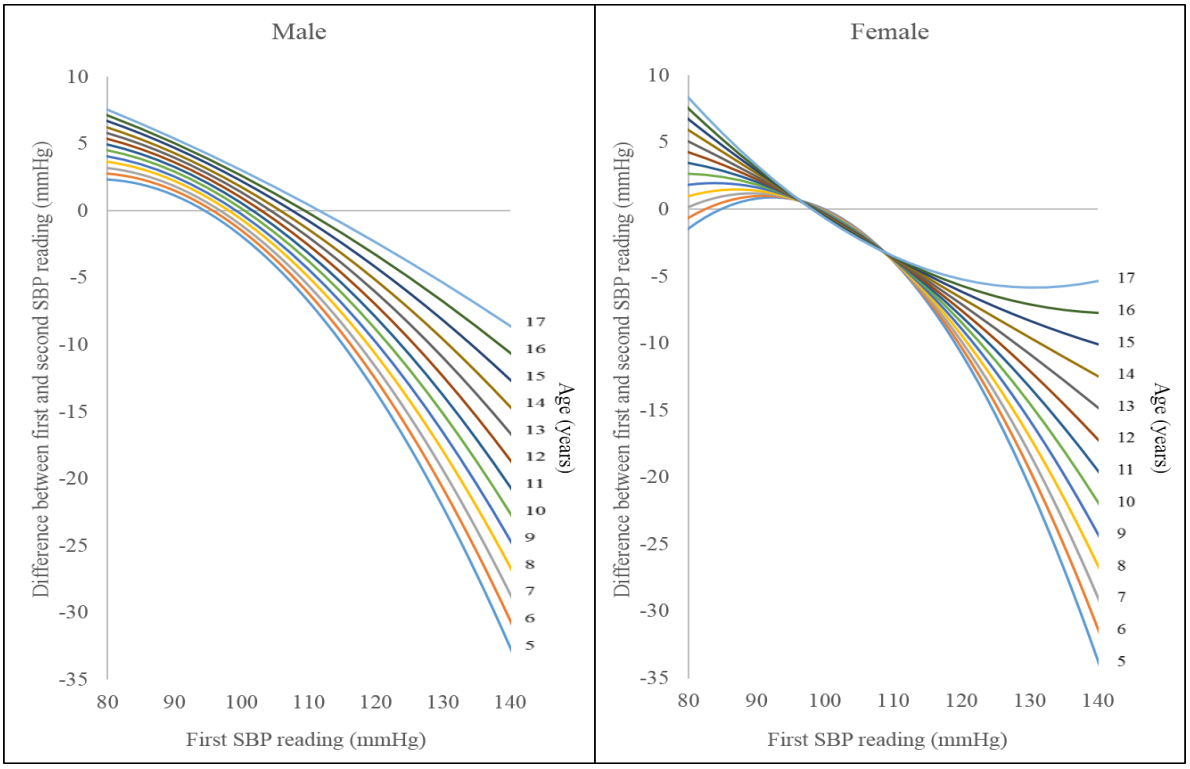

Figure 5. 1 Modifying effect of age on the relationship of the difference between first and second systolic BP (SBP) readings and the level of the first SBP reading among female and male children and adolescents.

5.5 Discussion

Although BP is thought to be highly variable in childhood, data assessing the magnitude of within-visit BPV among youth are limited. Moreover, BP is thought to systematically decrease over consecutive measurements due to a reduction in anxiety related to the measurement process. The main findings of this study indicate that within-visit BPV among youth is highly affected by age, such that older children had smaller changes between consecutive BP measures, at any given BP level, in comparison to younger children. Secondly, assumptions that BP decreases over consecutive measurements is misleading because BP was observed to increase in about one third of those tested, whereas it did not change in 10% of participants in this large representative population of young people. Overall, these findings have implications regarding the accuracy of BP measurement and hypertension diagnosis in children and adolescents.

To our knowledge, this is the first study to report within-visit BPV indices such as the absolute change in BP over consecutive measurements and the overall BPV among three BP measurements. In this population, average ΔSBP (taking into account the direction of change) was -2.4 mmHg, whereas average ΔSBP_{ABS} was 6.7 mmHg and average SBPV was 8.2 %. The ΔSBP appears to be misleadingly small (-2.4 mmHg) owing to 10% of the population having no change and 32% having an increase from SBP1 to SBP2. The most meaningful measures in terms of magnitude of BP change is best quantified using the absolute BP change or the overall BP variability (defined in this analysis as the coefficient of variation; a measure of the distribution of values around the mean). To our knowledge, only one study has previously reported ΔSBP and this was among 10-19 year old adolescents (SBP change from SBP1 to SBP2: -3.8 mmHg and SBP change from SBP2 to SBP3: -1.2 mmHg);[137] however, as said above, based on this study's findings which show that BP will not systematically decrease over consecutive readings, these measures do not allow for the best indication of the full magnitude of ΔSBP. Within-visit BP variance in children was firstly studied in 1987 by Rosner et al.[126] among 780 children aged 8-18 years. The within-visit variance components (defined as the squared deviation from the mean value [σ^2]) were derived from a nested random effects model of three BP measures repeated at 4 visits (assessed using a sphygmomanometer), and although the level and dispersion of BP data in the context of BP assessment would be easier interpreted using either standard deviation or coefficient of variation, these data provided the first evidence of a highly variable BP profile in childhood.

Although it is believed that children have greater BPV in comparison to adults, there are limited data to support this notion. Rosner et al.[126] compared within-visit BP variance components among 780 children with previously published results among 434 adults and concluded that within-visit BP variance was considerably higher in childhood. However, this comparison was made from two different studies with the adult population having a narrow age range (30-39

years). Our group recently investigated the impact of BP level and age on within-visit BPV indices among >20,000 adults aged 18-85 years using the same population survey methodology used in this analysis.[131] Compared with children, adults had smaller changes from SBP1 to SBP2 and smaller overall variability (ΔSBP_{ABS} = 4.9 mmHg; ΔSBP = -1.7 mmHg and SBPV = 6.56 %).[131] This lower BPV among adults (despite considerably higher BP levels) may be a normal physiological process of cardiovascular ageing, perhaps reflecting diminished minute-to-minute BP regulatory ability.[131] Interestingly, the findings of this current study suggest that even in youth there is an adverse impact of higher BP on the normal inverse relationship between BPV and age.

Remarkably, the proportion of adults with either a decrease, increase or no change from SBP1 to SBP2 among the AHS 2011-2013 survey was very similar to that observed among young people (adults; 56%, 37% and 7% respectively, children; 58%, 32% and 10% respectively). Although we cannot speculate as to the reasons underlying the direction of BP changes over consecutive measurements, it seems unlikely to be driven solely by a participant's stress levels if one takes into account the high proportion of individuals with an increase or no change from SBP1 to SBP2. Additionally, the younger population, being less exposed to the BP measurement process as compared to adults, may be expected to have a different stress response and therefore have a different direction of SBP change if it was driven by the stress level, but this was not the case. Lastly, our findings among both youthful and adult populations are similar to previously published data which assessed within-visit home BPV and found a decrease in SBP among 60% of the population, but an increase among 30%, and no change among 10% of the population.[138] Out-of-clinic BP assessment, such as home BP, minimizes the procedural stress and alerting response to the measurement,[80] therefore the similar patterns observed in SBP change in both home and clinic BP, support the unlikelihood of this being only the result of anxiety or stress.

Importantly, the prevalence of hypertension depends on the number of BP readings used for its estimation.[131] Although repeated measurements are recommended for BP assessment in children and adolescents, the exact number is not specified. Additionally, the number of measurements vary across health care providers[139] but also across research studies reporting prevalence of hypertension. The number of readings used for BP assessment vary from two[140-142] and three BP,[143-146] to four[147] readings. Studies have also used the average of second and third readings discarding the first one,[148] or recorded the lower of three readings.[149] However, with the difference in BP across percentiles of BP classification being very small (3-4 mmHg), especially between the 90th (pre-hypertension stage) and 95th (hypertension stage) percentiles, the assessment and classification of high BP will substantially vary depending the number of readings, particularly with increased BPV. Indeed, depending on the number of readings used for BP assessment, Wirix et al. reported a range of hypertension prevalence from 32.5% to 12.4% among overweight children, and from 21.2% to 4.6% among non-overweight children (using either the first BP only, the mean of two BP readings, the mean of three BP readings, the median of three BP readings or the lowest of three BP readings).[149] Our findings, in the context of data on hypertension prevalence among children and adolescents, underscore the need to use out-of-clinic BP monitoring to confirm clinic BP readings, as well as the need for more accurate clinic BP measurement protocols (e.g. automated, unobserved BP).[150]

Strengths of this study include the survey design and large sample size which allow for representative estimates of a general population. Additionally, this is the first study to provide estimates of within-visit BPV which are directly comparable to adults, using results derived from the same assessment protocol in both children and adults.[131] On the other hand, a limitation arises from the BP measurement process which was undertaken by trained non-clinicians in the home rather than under strict laboratory conditions or by clinicians. These different approaches to study design may have affected the level of BP, for example, by

lessening the white coat effect had readings been taken by clinicians in the office. However, the effect on BPV cannot be predicted. Lastly, analysis using all three BP readings has been performed on an incomplete dataset because a third reading was only taken if there was ≥10 mmHg difference between the first and second BP readings, and was not a predefined methodological protocol. Thus, the results of analyses which included all three BP measures could be more relevant to populations with increased BP variability.

In conclusion, within-visit BPV is highly affected by age in children and adolescents, with older children having smaller changes for any given BP level, in comparison to younger children. Employing the same clinic BP assessment protocols in children and adolescents as in adults may not be ideal due to physiological differences in short-term BP behavior over consecutive measurements. Additionally, it is important to keep in mind that BP will not drop with repeated measurements for a sizeable proportion of the population, and thus BP measurement protocols should allow for the detection of a possible increase in BP. As precision in BP measurement is critical;[151] the need for better measurement protocols is underscored. Further research should aim in determining the optimal required number of readings for an accurate clinic BP assessment; however, until reliable clinic BP methods are established, out-of-clinic BP measures (i.e. home or 24-hour BP) should be used to confirm diagnosis.

5.6 Contribution of chapter 5 to thesis aims

Chapter 5 (study 1), represents the first study to examine the direction and magnitude of within-visits BP fluctuations over consecutive measurements, as well as factors affecting these fluctuations, in a nationally representative sample of more than 3,000 children and adolescents from the Australian Health Survey. The key findings were that BP is highly variable. However, the magnitude of change in BP over consecutive measurement was greater among children and adolescents with higher BP levels, but lower with older age. Moreover, in contrast to

expectations that BP should always drop over consecutive measurements, systolic BP increased on repeat measurement among a significant proportion of the population. These observations are highly relevant to the correct clinical determination of BP control among children and adolescents.

Chapter 6

Effect of Vitamin D Supplementation on Aortic Stiffness and Arterial Hemodynamics in People With Osteoarthritis and Vitamin D Deficiency*

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Veloudi P., Blizzard L., Ding CH., Cicuttini FM., Jin X., Wluka AE., Winzenberg T., Jones G., Sharman JE. Effect of Vitamin D Supplementation on Aortic Stiffness and Arterial Hemodynamics in People With Osteoarthritis and Vitamin D Deficiency.

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6.1 Abstract

Background. Increased aortic stiffness, peripheral and central blood pressure (BP) predict cardiovascular events and mortality. Observational studies report inverse associations between vitamin D levels, aortic stiffness and BP, but there are limited intervention data.

Objectives. This study aimed to determine the effect of vitamin D supplementation on aortic stiffness, peripheral and central BP indices, as well as visit-to-visit BP variability (BPV).

Methods. In a double-blind, placebo-controlled trial, 241 individuals (mean[SD] age 63[7] years, 49% female) with vitamin D deficiency (43.12[12.24] nmol/L) and knee osteoarthritis were randomized to 12-months vitamin D 50,000 IU/month (n=118) or matching placebo (n=123). Our primary outcomes were aPWV (carotid-femoral pulse wave velocity; aPWV), peripheral BP and central BP and were measured at baseline, 6- and 12-months. Visit-to-visit BPV was calculated from three visits over 12-months.

Findings. Vitamin D supplementation significantly increased serum vitamin D compared with placebo (45.10 [95%CI 40.20 to 49.93] vs 7.99 [95%CI 4.32 to 11.66] nmol/l; p<0.001). However, relative to placebo there were no significant differences for changes in aPWV (-0.10 [95% CI -0.47 to 0.26] vs 0.05 [95%CI -0.33 to 0.42] m/s; p=0.56]), peripheral systolic BP (-3.00 [95%CI -5.60 to -0.40] vs -2.94 [95%CI -5.59 to -0.30] mmHg; p=0.98) or any other peripheral or central BP indices, or visit-to-visit BPV (all p>0.05).

Conclusions. Despite many observational studies suggesting that vitamin D supplementation could be useful for improving aortic stiffness and BP, 12-months intervention yielded no improvement in older people with vitamin D deficiency and osteoarthritis. Registration number: ClinicalTrials.govidentifier: NCT01176344.

6.2 Introduction

Increased aortic stiffness [152], peripheral blood pressure (BP) [153] and central hemodynamic parameters [154] (such as augmentation index [AIx]), independently predict cardiovascular events and all-cause mortality. Moreover, BP variability (BPV) has recently been shown to be an independent predictor of cardiovascular risk. [155]. Observational studies show detrimental associations between aortic stiffness [156], peripheral BP [157] and central BP [158] and low vitamin D levels among different patient populations. It has also been shown that low vitamin D may adversely affect BPV via increased large artery stiffness [76]. Experimental data suggest that vitamin D could directly benefit the vasculature [159] in addition to acting as a negative regulator of the renin-angiotensin system to influence BP control [160], and thereby change large artery stiffness [53].

Data from intervention studies assessing the effects of vitamin D on aortic stiffness and BP indices are sparse and inconclusive. A number of randomized-controlled clinical trials testing the effect on aortic stiffness (as measured by aortic pulse wave velocity [aPWV], the current 'gold standard' measure) [57] have recently been published [161-164] but results are equivocal. Probable reasons for this are study design issues, including small sample size [161,164] and short-term duration (<6 months) [162,163]. Similar problems of short-term intervention [165-168], low vitamin D dose [169] or small sample sizes [170-173] are evident in studies investigating the effect of vitamin D supplementation on peripheral BP (only two studies of appropriate dose, sample size and intervention period found no effect of vitamin D supplementation on peripheral BP [174],[175]. Effectiveness of vitamin D supplementation on central hemodynamics is unknown, with only small, short-term (<4 months) interventions reporting no effect on central BP or AIx in postmenopausal women [162], or patients with peripheral arterial disease [176] or chronic kidney disease [164]. There are no vitamin D intervention studies targeting visit-to-visit BPV.

People with osteoarthritis (OA) are more likely to be vitamin D deficient [177] and this may increase progression of knee OA [178]. OA is associated with increased propensity for macrovascular atherosclerotic disease (carotid artery intima media thickness and plaque severity, and coronary artery calcification) [179,180] and increased large artery wall thickness [181]. The progression of OA has also been shown to independently relate to accumulation of cardiovascular risk factors, including hypertension [182]. Mechanisms that contribute to OA (i.e. inflammation and oxidative stress) may overlap with development of adverse arterial changes leading to large artery stiffness and increased BP [183]. Furthermore, medications for OA can increase cardiovascular risk through BP raising effects [184] and this can have major health consequences for which treating clinicians need to weigh risk versus benefit [185]. Taken altogether, people with OA represent a population enriched with vascular risk factors that may be amenable to benefit with treatment from vitamin D supplementation. This current study is a sub-study of a trial investigating the effect of vitamin D supplementation on musculoskeletal outcomes among older people with vitamin D deficiency and osteoarthritis [186]. Its aim was to determine the effects of vitamin D supplementation in this population on aPWV, peripheral and central BP measures, including BPV.

6.3 Methods

Study design. This was a sub-study of the VItamin D Effects on Osteoarthritis, (VIDEO) study, a randomized, double-blind, placebo-controlled trial, the rationale and design of which has been published [186]. Briefly, the rationale of a vitamin D intervention targeting osteoarthritis among older people is based on the potential benefits of vitamin D on knee structure, bone and muscle health in a population where knee osteoarthritis is common and vitamin D deficiency prevalence reaches 30% or more [186]. The primary end-points of the VIDEO study were knee cartilage volume measured by magnetic resonance imaging (MRI) and Western Ontario and McMaster Universities Index of Osteoarthritis (WOMAC) score. We considered that the

VIDEO study design presented an opportunity to determine the effect of vitamin D on BP and vascular endpoints. The only major protocol change of the sub-study was failure to measure 24 hour ambulatory BP due to insufficient resources. The complete study was performed between 2010 and 2013 and there were 400 participants allocated to either intervention or placebo from two major centres (Hobart, Tasmania and Melbourne, Victoria). Sub-study data relating to this paper were collected at the Hobart site only. The study was approved by the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040).

Participants. Exclusion/inclusion criteria have been described previously in detail [186]. Briefly, inclusion criteria were: having knee osteoarthritis; aged 50 – 79 years; relatively good health, with a score of 0 to 2 on a 5-point Likert scale (0 indicating very good health and 4 indicating very poor health); serum vitamin D levels > 12.5 nmol/L and < 60 nmol/L; and knee pain. Exclusion criteria were: severe knee osteoarthritis, severe knee pain on standing or significant knee injury; any contradiction to having MRI; hypersensitivity to vitamin D; any condition possibly affecting oral drug absorption; rheumatoid or psoriatic arthritis, lupus or cancer; severe cardiac or renal impairment; and history of taking vitamin D supplements within the previous 30 days. All participants provided written, informed consent.

Randomization and masking. Allocation was based on computer-generated random numbers in a ratio of 1:1. Allocation was double-blind and concealment was achieved using a secure central automated allocation procedure blinded to all investigators. Statistical analyses were performed by a person (PV) blinded to allocation until after completion of all data analysis.

Procedures. Participants were randomized to receive intervention (monthly capsule of 50,000 IU [1.25 mg] of vitamin D3 [cholecalciferol]) or an identical inert placebo. aPWV, peripheral and central BP were measured at baseline, 6 and 12 months. All measures were recorded in a quiet, temperature-controlled room. Safety and adverse events (AEs) were assessed at each

follow-up visit and information related to cause or intensity of the AEs as well as hospitalization admission was recorded.

Outcomes. Our primary outcomes were aPWV, peripheral BP and central BP, specified a priori before commencing recruitment [186], with visit-to-visit BPV added as a hypothesis generating analysis on the basis of the clinical relevance of stabilizing BPV [187], which may be possible with improved vascular stiffness [188]. Supine aPWV was assessed in duplicate using electrocardiogram-gated carotid to femoral tonometry readings according to guidelines [57] using customized equipment (SphygmoCor 8.0, AtCor Medical, Sydney, NSW). Brachial BP was measured by the average of two consecutive BP readings taken after 5 minutes of seated rest using a validated automatic device (Omron HEM-907; OMRON Europe B.V. (OMCE), Hoofddorp, The Netherlands). Measurements were recorded using an appropriate sized cuff, with the participants' arm supported at heart level, with their back supported and feet flat on the floor. Radial applanation tonometry was used to measure central BP (SphygmoCor 8.1, AtCor Medical, Sydney, Australia), immediately after each peripheral BP measurement. Central BP was derived from the aortic pressure waveform which was reconstructed using a validated generalized transfer function applied to the radial artery waveform [189] and the average of two readings was used for analysis. Augmentation pressure (AP) was calculated from the aortic pressure waveform as P2 (late systolic peak) – P1 (early systolic peak) and indexed to pulse pressure (PP) for calculation of AIx. Since AIx is heart rate-dependent, this variable was also corrected to a standard heart rate of 75 beats per minute (AIx@75). PP was calculated as the difference between systolic BP and diastolic BP. PP amplification was defined as peripheral PP/central PP. The mean of peripheral systolic, diastolic, PP and mean arterial pressure (MAP) as well as central systolic BP from each visit over 12 months was used to calculate visit-to-visit BPV for each variable. This was determined from the standard deviation (SD) around the

mean, as well as the coefficient of variation (CV; [SD/mean]x100) for the BP values among those participants who completed all three visits.

Serum vitamin D levels at baseline and 3 months were measured from serum samples treated initially with acetronitrile to rapidly extract 25-hydroxyvitamin D [25-(OH)D]. 25-(OH)D was then assayed utilizing a Liquid Phase radioimmunoassay (Immunodiagnostics Systems Ltd, Boldon, Tyne & Wear, UK).

Statistical analysis. We sought to recruit as many participants as possible from within VIDEO, with a minimum of 100 participants in each arm allowing for a between-group clinically significant detectable difference of at least 0.5 m/s for aPWV (with the predicted standard deviation as 1.6 [190] [α=0.05, β=0.20]). Based on our previous data [191], this sample size was predicted to detect a between-group difference of at least 4.5 mmHg for peripheral and central systolic BP. The intervention effect on outcome measures was assessed based on the assigned treatment at randomization on all participants who were randomized, regardless of adherence or study retention (intention-to-treat approach). Between-group differences in the change in outcome measures over each visit during the 12 months follow up were assessed using mixed-effects linear regression models with random intercepts. In keeping with published evidence [53], the changes in aPWV were also estimated after adjusting for the changes in MAP in order to account for the known contribution of MAP to aPWV. The maximum likelihood approach was applied to address missing data uisng the 'xtmixed' Stata command. Student t-tests were used to compare visit-to-visit BPV between groups. Sensitivity analyses were performed in order to test the potential effects of deviation from the protocol (also analyzed per protocol) and different definitions to delineate abnormality of outcome variables (also analyzed using different cut-off values; *post hoc* analyses). The *post-hoc* analysis model for the changes in aPWV was also adjusted for the change in MAP over time. A p value <0.05 was considered

statistically significant. All data were analyzed using Stata 12.1 (StataCorp, College Station, Tx). The trial was registered (ClinicalTrials.govidentifier: NCT01176344).

6.4 Results

Figure 6. 1 depicts the trial profile. From 422 people screened for eligibility, 265 were randomly assigned to either intervention matching placebo. 24 individuals were either lost to follow-up or discontinued the intervention. 241 participants were included in the analysis of aPWV and BP measures (mean [SD] age 63[7] years, 49% female); 118 were assigned to intervention and 123 were assigned to matching placebo. Participant characteristics are described in Table 6. 1. Characteristics of participants in each group were similar other than participants in the placebo group having a slightly higher prevalence of high cholesterol (41.2% vs 47.0%). There were no between-group differences for any other characteristic, including age, sex or body mass index (BMI). Almost half of the participants in each group self-reported high BP. A small percentage in each group self-reported a previous history of myocardial infarction or stroke. The percentage of missing data from the total population for aPWV and BP measures at baseline was 2.1% and 5.4%, at month 6 was 15.5% and 9.6% and at month 12 was 18.0% and 11.7% respectively. For all participants, baseline serum vitamin D was 43.1(12.2 nmol/L); there were no differences between the intervention and the placebo group. Serum vitamin D increased significantly more with intervention compared to placebo (45.10 [95%CI 40.20 to 49.93] vs 7.99 [95%CI 4.32 to 11.66] nmol/L; $p<0.001$).

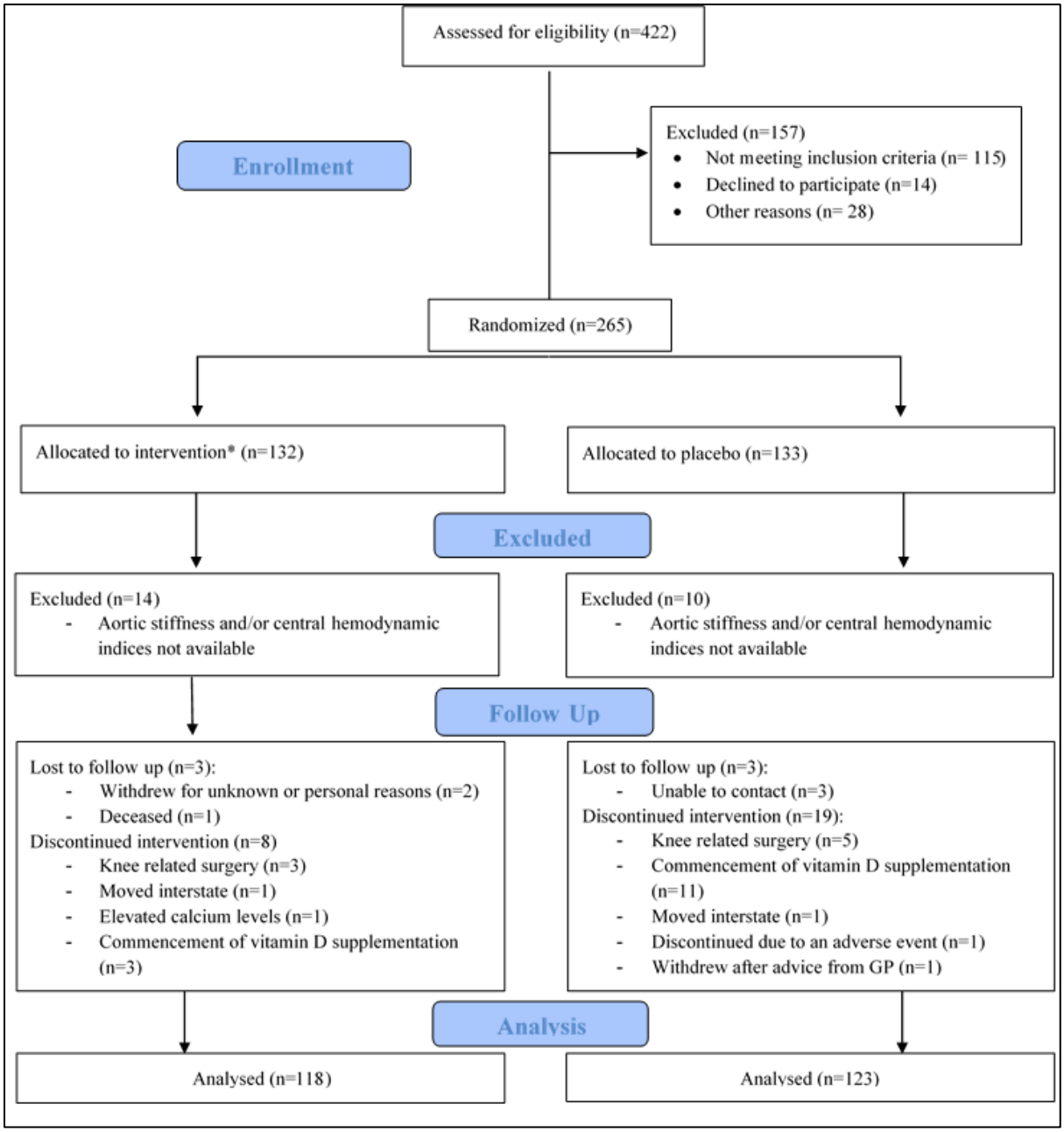

Figure 6. 1 Flow of the study participants.

*Intervention: vitamin D 50,000 IU/month

Table 6. 1 Baseline demographics and clinical characteristics of study participants

Participant clinical characteristics	**Intervention (n=118)**	**Placebo (n=123)**
Mean age (years)	63.3(7.2)	62.4(7.3)
Body mass index (kg/m^2)	29.4(5.2)	29.6(4.4)
Female, %(n)	50.4(59)	47.2(58)
Participant-reported, %(n)		
High blood pressure	50.9(60)	48.0(60)
High cholesterol	41.2(49)	47.0(57)
Type II diabetes mellitus	7.0(8)	7.6(9)
Asthma	21.1(25)	17.6(22)
Myocardial infarction	4.4(5)	1.7(2)
Stroke	1.8(2)	2.5(3)
Serum vitamin D concentration (nmol/L)	42.4(11.8)	43.8(12.6)
Aortic stiffness		
Aortic pulse wave velocity (m/s)	9.2(2.5)	9.1(2.0)
Peripheral blood pressure (mmHg)		
Systolic blood pressure	128.5(15.4)	128.2(15.0)
Diastolic blood pressure	73.2(8.6)	72.8(9.7)
Mean aortic pressure	95.3(10.4)	94.9(10.5)
Pulse pressure	54.5(10.8)	55.4(12.4)
Central blood pressure (mmHg)		
Systolic blood pressure	117.1(15.2)	117.1(15.0)
Pulse pressure	42.4(10.3)	43.0(11.7)
Augmentation pressure	11.3(5.9)	11.1(6.9)
Augmentation index (%)	20.8(8.2)	21.5(8.8)

Data presented as mean(SD) unless otherwise stated.

There was no significant between-group difference in change in aPWV; with a decrease of 0.22 m/s for the intervention group and a slight increase of 0.06 m/s for the placebo group at follow up (Table 6. 2). The difference between the groups attenuated when the model was adjusted for the changes in MAP (-0.10 [95% CI -0.47 to 0.26] vs 0.05 [95%CI -0.33 to 0.42] m/s; p=0.56). Unadjusted post-hoc analysis confined to only those participants with high baseline aPWV according to guidelines[45] (>10 m/s; intervention n=34 vs placebo n=33) showed a near significant effect of intervention (intervention; -1.77 [95%CI -2.57 to -0.97] vs placebo; -0.72 [95%CI -1.50 to 0.07] m/s; p=0.065). However, this difference attenuated when adjusted for the change in MAP (p=0.14). Results of per protocol analyses were similar.

Table 6. 2 Changes over 12 months in aortic stiffness, peripheral and central hemodynamic parameters by study arm

Variable	Intervention (n=118)	Placebo (n=123)	P*
aPWV (m/s)	-0.26(-0.62,0.10)†	0.06(-0.30,0.43)	0.22
Peripheral SBP (mmHg)	-3.00(-5.60,-0.40)	-2.94(-5.59,-0.30)	0.98
Peripheral DBP (mmHg)	-1.47(-3.00,0.06)	-0.53(-2.09,1.03)	0.40
MAP (mmHg)	-2.08(-3.93,-0.24)	-1.48(-3.36,0.39)	0.66
Peripheral PP(mmHg)	-2.48(-4.35,-0.61)	-0.95(-2.76,0.85)	0.25
Central SBP (mmHg)	-2.77(-5.42,-0.11)	-2.90(-5.59,-0.22)	0.94
AP (mmHg)	-0.84(-1.86,0.17)	-0.16(-1.17,0.86)	0.35
Central PP (mmHg)	-2.10(-4.03,-0.18)	-0.93(-2.81,0.95)	0.40
AIx@75	0.54(-1.75,0.68)	0.77(-0.45,1.99)	0.14

*P value for the interaction between group and time, as derived from mixed-effects linear regression.
†Beta coefficient (95% confidence interval) (all such values).
aPWV = aortic stiffness; SBP = systolic blood pressure; DBP = diastolic blood pressure; MAP = mean arterial pressure; PP = pulse pressure; AP = augmentation pressure; AIx@75 = augmentation index adjusted for heart rate; PPA = PP amplification.

The changes in peripheral systolic BP did not differ significantly between intervention and placebo (-3.00 [95%CI -5.60 to -0.40] vs -2.94 [-5.59 to -0.30 mmHg; p=0.98, Table 6. 2). Similarly, no significant between-group differences were observed for changes in peripheral systolic BP when analysis was repeated for participants with baseline systolic BP≥140 mmHg (intervention n=33 vs placebo n=34, p=0.37). No significant between-group differences were observed for changes in any other peripheral or central BP measure or PP amplification (Table 6. 2). Analysis of the central BP measures was repeated after recalibration of the radial pressure waveforms with mean arterial pressure (MAP; derived by 40% form factor) and diastolic BP; however, results were similar to those where central BP was derived by calibration using brachial systolic BP and diastolic BP (data not shown). None of the above findings were altered when analyzed per protocol.

BPV could be assessed in 194 people who completed all three visits. There were no significant between-group differences in the visit-to-visit BPV in either peripheral or central BP measures (Table 6. 3; p>0.17 for all).

Table 6. 3 Between-group comparison of visit-to-visit variability in BP indices

Variable	**Intervention (n=102)**	**Placebo (n=92)**	**P**
Peripheral systolic BP SD	8.64(5.40)	7.67(5.92)	0.23
Peripheral systolic BP CV	6.90(4.49)	6.07(4.67)	0.21
Peripheral diastolic BP SD	5.10(2.87)	4.69(2.99)	0.36
Peripheral diastolic BP CV	7.16(4.14)	6.52(4.08)	0.28
Mean aortic pressure SD	6.16(3.53)	5.47(3.95)	0.20
Mean aortic pressure CV	6.64(3.93)	5.84(4.20)	0.17
Central systolic BP SD	8.31(5.09)	7.96(6.66)	0.67
Central systolic BP CV	7.21(4.39)	6.91(5.34)	0.67

Data presented as mean(SD).
BP = blood pressure; SD = standard deviation; CV = coefficient of variation.

24 participants in the intervention group and 20 participants in the placebo group reported one or more AEs during the 12-months follow-up period (20.34% and 16.26%, respectively; p=0.43). The total AEs reported were 64; 35 in the intervention group and 29 in the placebo group. 5.71% of the total AEs reported in the intervention group and 6.90% of the total AEs in the placebo group were classified as serious AEs (p=0.86). Table 6. 4 shows the classification of all serious and non-serious AEs per group.

Table 6. 4 Adverse events among participants

	Intervention (n=118)	**Placebo (n=123)**
Serious adverse events (n=20)		
Death (non-cardiovascular)	0.85 (1)	0.00(0)
Cancer	3.39(4)	1.63(2)
Myocardial infarction	0.00(0)	0.81(1)
Coronary artery bypass	0.00(0)	0.81(1)
Cerebrovascular accident	0.85(1)	0.00(0)
Hospitalisation after suspected stroke	0.85(1)	0.00(0)
Severe infection	0.00(0)	1.63(2)
Nephrolithiasis	0.85(1)	0.00(0)
Gastrointestinal disorder	0.85(1)	0.00(0)
Major depression	1.69(2)	0.00(0)
Hospitalisation after a fall	1.69(2)	0.00(0)
Total	11.02(13)	4.88(6)
Non-serious adverse events (n=97)		
Hypercalcaemia	5.08(6)	1.63(2)
Hematologic disorder	0.00(0)	0.81(1)
Nephrolithiasis	0.00(0)	0.81(1)
Falls	1.69(2)	0.00(0)
Hyperparathyroidism	0.85(1)	0.00(0)
Renal	2.54(3)	0.00(0)
Urinary	0.85(1)	0.81(1)
Neurological	4.24(5)	2.44(3)
Gastrointestinal	3.39(4)	4.07(5)
Musculoskeletal	0.85(1)	0.81(1)
Respiratory	1.69(2)	1.63(2)
Ocular/visual	1.69(2)	1.63(2)
Infection	5.93(7)	2.44(3)
Cardiac arrhythmias	2.54(3)	0.00(0)
Chest pain	2.54(3)	4.07(5)
Pain	6.78(8)	2.44(4)
Allergy/immunology	0.00(0)	1.63(2)
Other events*	8.47(10)	5.69(7)
Total	49.15(58)	31.71(39)

Data are presented as %(n).
*Includes: headache, lethargy, flu symptoms and other events.

6.5 Discussion

This study provides definitive evidence that vitamin D supplementation does not improve large artery stiffness or BP control in a vitamin D deficient older aged population enriched with risk factors to identify an effect. It is the first study that has had adequate sample size to detect clinically important effects on aPWV and central BP outcomes, each of which representing major independent risk factors for cardiovascular events and mortality [152,154]. Our findings also add novel information on visit-to-visit BPV and confirm recent work showing no effect of vitamin D on peripheral BP [167,174]. Importantly, the substantial increase in serum vitamin D levels in the intervention group ensures that the lack of effect is not due to poor compliance or inadequate dose. Altogether, the difference in the changes in aPWV and BP measures were neither statistically significant nor likely to be clinically important.

The previously reported positive effects of vitamin D on cardiovascular outcomes in observational data could have been influenced by the relationship of vitamin D with other factors. For example, low physical activity [192] or obesity [193] could be associated with low vitamin D levels, but be the causative factors with respect to increased cardiovascular risk (i.e. increased risk of diabetes). This is the longest vitamin D intervention trial assessing the effect on aPWV and the result is concordant with previous negative studies of considerably shorter duration [162-164]. Importantly, when accounting for the effect of BP on aPWV [53] by adjusting for the changes in MAP, the treatment effect decreased from -0.26 to -0.10 m/s, which suggests that the initially unadjusted treatment effect on aPWV was not independent of BP changes. Additionally, analysis restricted to those participants with high baseline aPWV according to guidelines (>10 m/s) and adjusted for the change in MAP, showed no significant differences among the groups. This study is also the longest intervention trial to test the effects of vitamin D supplementation on central BP indices, and the results confirm negative effects in smaller studies of select patient populations [176], [162], [164].

Strengths of our study include the randomised, double-blind design, as well as an appropriately lengthy duration and the effectiveness of intervention in increasing serum vitamin D above deficiency levels. Moreover, the patient population was replete with clinical characteristics amenable to showing an effect of vitamin D, and this is the only study appropriately powered to observe clinically significant differences in aPWV. The study limitations include the possibility that participants could have had increases in endogenous vitamin D through higher exposure to sunlight or artificial ultraviolent radiation treatment, but this is unlikely to affect or results given the double-blind randomisation protocol and the fact that serum vitamin D levels were substantially higher in the supplemented group. Secondly, we did not measure 24 hour ambulatory BP, which is a stronger marker for cardiovascular outcomes than clinic BP, and thus cannot exclude a differential impact on this variable. However, vitamin D has been shown to be ineffective for reducing 24 hour ambulatory BP in patients with isolated systolic hypertension [175]. Additionally, the findings pertaining visit-to-visit BPV may have been affected by the number of BP readings used in this analysis (three BP readings). Nevertheless, there are no guidelines as to how many numbers are needed for accurate quantification of visit-to-visit BPV. Lastly, as a sub-study of a larger trial specifically designed to determine the effects of vitamin D among people with osteoarthritis and vitamin D deficiency, the findings may not be generalizable to people outside this selection criteria. However, as alluded above, the treatment effects would be expected to be smaller in a population without vitamin D deficiency. Similarly, as there is evidence suggesting that osteoarthritis is associated with increased cardiovascular risk [194,195], the effect of a vitamin D treatment in a population with osteoarthritis becomes more meaningful rather than in a general population.

Conclusions. In conclusion, this study found no effect of vitamin D supplementation on aPWV, BP, or BPV among older people with vitamin D deficiency and osteoarthritis. Despite a plethora of observational data supporting a relationship between vitamin D and

cardiovascular health via pathways involving BP and large artery stiffness, evidence from our high quality randomised-controlled clinical trial and other existing trials do not support the use of vitamin D supplementation as an intervention to improve these endpoints. The previously documented associations between vitamin D, aPWV and BP are likely to be epiphenomena rather than causative and vitamin D supplementation for these aspects of cardiovascular health cannot be recommended.

6.1 Contribution of chapter 6 to thesis aims

Chapter 6 (study 6) represents the first study to investigate the efficacy of targeted central arterial stiffness lowering interventions on long-term BPV indices (both brachial and central BPV indices). Study 6 explored the effect of vitamin D supplementation on long-term BPV, aortic stiffness, peripheral and central BP indices. Despite many observational studies suggesting that vitamin D supplementation could be useful for improving cardiovascular health, 12-months intervention yielded no improvement in long-term BPV or mean BP levels of aortic stiffness in older people with vitamin D deficiency and osteoarthritis.

Summary and future directions

This research project has shown that the methodology of measuring and quantifying BPV could significantly affect the magnitude of BPV itself and this could have various clinical implications. Contrary to expectations, this thesis does not support the hypothesis that BPV is clinically important in patients with uncomplicated hypertension and lower cardiovascular risk. However, this work offers some evidence that BPV may be a potentially modifiable target for microvascular damage in high-risk populations. Moreover, this thesis shows unequivocal evidence that BPV could also affect the measurement and assessment of mean BP levels and therefore the classification of high BP; this could have major clinical consequences. Lastly, it has been shown that vitamin D supplementation was not an effective intervention in lowering long-term BPV.

In chapter 1, the methodology of measuring BPV was explored for the first time using a scoping review and the results indicate that certain methodological factors (i.e. the number of BP measurements or the duration of BP monitoring) as well as participants' age and mean BP level, could significantly affect BPV magnitude. Although methodological discrepancies and heterogeneity have been suggested to be among the reasons of conflicting results among studies,[10,11] there has never been a systematic approach to quantify their impact on BPV assessment. Therefore, these results could offer the foundations for standardizing the protocols and methodology of measuring BPV, which is necessary in order to compare results among studies but also to enable research integration into clinical practice. The abundance of studies investigating BPV in relation to various research questions (i.e. organ damage, antihypertensive treatment, physiological correlates of BPV) and the plethora of methodologies used across these studies have led to further confusion rather than clarity. An inevitable question is, as noted by Whittle;[25] "now what?" Should we measure BPV in clinical practice and if yes, how should we measure it? Based on the results of chapter 1, the next step would be to offer recommendations on how to assess each type of BPV based on methodological

factors that were outlined in this analysis, such as the number of BP readings or visits, or the duration of the monitoring which is required for the assessment. The recommendations should also clarify which type of data should be reported by studies. For example, most studies report the primary results using the standard deviation but the majority do not report average BPV levels. Within the scoping review, we recommended that studies should report average BPV levels using a metric with low computational complexity (i.e. coefficient of variation) to enable easier translation of results and integration in clinical practice; however, other metrics independent of the mean should also be used. Minimizing methodological heterogeneity would enable better comparison of the results and eventually it will allow systematic meta-analyses to draw stronger conclusions with respect to the measurement of BPV in clinical practice. At the same time, it is critical to identify age-specific cut-off values for risk stratification, taking into account the level of mean BP. The final step would be to establish reliable cut-off values based on standardized protocols that would allow the interpretation of evidence and enable translation into clinical practice.

The results of chapter 2 represent the first evidence of potential implication of short-term BPV in the pathophysiology of microvasculature, in patients with type II diabetes mellitus. Given that many scholars have highlighted the possibility of BPV being a modifiable target, especially in people with higher cardiovascular risk (i.e. individuals with type II diabetes mellitus), these results could have potential clinical implications. On the other hand, as this was a small, cross-sectional, post-hoc, hypothesis generating analysis, future research should explore the associations between BPV and microvascular pathophysiology in large-scale, longitudinal studies in order to determine causative relationships. Furthermore, it has been suggested that the pathophysiology of microvasculature could play a role in the relationship between macrovascular damage and BPV.[196] The hypothesis that BPV is dependent on large artery stiffness has not been unequivocally supported;[26,66,197] therefore, the discrepancies seen across

studies may reflect a more complex phenomenon representing interrelationships between the micro- and macrovasculature and BPV which needs to be further explored in appropriately large and well-designed studies. Findings from chapter 3 shed more light towards the association between BPV and the macrovasculature in a population with low to moderate cardiovascular risk.[198]

The results of chapter 3 represent the first study to show that longitudinal changes in BPV (short- and mid-term) as well as long-term BPV were not predictive of organ damage (heart structure and macrovascular markers, such as aortic stiffness) in patients with uncomplicated hypertension and low to moderate cardiovascular risk. Advancing the evidence around the hypothesis that BPV is associated with large artery stiffness, these results may indicate that some organs are less susceptible to BP fluctuations than other (i.e. microvasculature versus microvasculature or heart versus eyes or brain). Alternatively, these results are in alignment with suggestions that BPV could offer prognostic information, over and above that of mean BP levels, in individuals with higher rather than lower cardiovascular risk. Nevertheless, the results of chapter 3 represent the first study to examine the effect of changes in the three main types of BPV on changes in heart structure and aortic stiffness over a one year follow-up period, in this population. This is extremely important as it addresses one of the main research gaps in the current literature around BPV; whether it is short-, mid- or long-term BPV that correlates with organ damage indices? This research question stems from the hypothesis that different types of BPV may represent a different pathophysiology;[51] therefore, correlating to different organ damage indices. However, further research needs to explore the changes in different BPV and changes in organ damage markers in populations with high cardiovascular risk in order to determine whether certain BPV types are superior than other types in predicting certain organ damage, or cardiovascular events and mortality. Furthermore, although this study was the first one to explore longitudinal associations between BPV and organ damage, it may be argued that

a longer follow-up duration (>1 year) was needed to draw solid conclusions as to whether BPV changes affect organ damage. Therefore, future studies should address these issues using follow-up periods longer than 1 year. Lastly, future research should also investigate longitudinal associations between changes in BPV and changes in other organ damage markers (i.e. kidney dysfunction), in order to clearly establish whether there is a causative relationship between BPV and the wider spectrum of organ damage. Nevertheless, the results support the view that clinical practice should remain focused on mean BP levels for patient management decisions, until further evidence can be derived.

Chapter 4 represents the first analysis investigating the impact of very short-term BPV (within-visit BP fluctuations) on hypertension classification and examined the magnitude and direction of change in BP over consecutive measures in a nationally representative sample of more than 20,000 participants from the Australian Health Survey. The principal goal was to determine how the changes in BP over consecutive measures may impact on the diagnosis of hypertension according to international guidelines. The major novel findings were contrary to anecdotal belief that BP will drop over consecutive measurements, and that the first BP is almost always higher than next measure due to an alarm reaction. Indeed, we found that BP may increase over consecutive measurements in a sizeable percentage of the population. Importantly, the magnitude of BP change was highly age- and BP-dependent, and the variability between measures resulted in significant diagnostic reclassification of hypertension across international guidelines. The clinical and financial implications of these findings are potentially major, taken into account that clinic BP measurement remains the most common method for hypertension diagnosis among epidemiological studies, but also in estimating prevalence of hypertension at national levels. It has been estimated that even a 5 mmHg overestimation or underestimation of BP levels could result in up to 21 million people being falsely diagnosed with hypertension or up to 27 million people misdiagnosed as normotensive in the United States.[82] The need for

evidence-based recommendations on how to measure clinic BP with minimal loss of clinical information has also been reinforced by the results of a recent high-profile clinical study (Systolic Blood Pressure International Trial; SPRINT).[199,200] SPRINT used a clinic BP measurement protocol based on a series of automated, unobserved BP readings; a method which minimizes the white-coat effect on mean BP levels. The conclusion of the study was that lowering systolic BP level below 120 mmHg as compared to the commonly used 140 mmHg target, reduces cardiovascular risk.[201] However, the question – which is highly relevant to the results of this study - was whether one could compare a target of 120 mmHg measured using an automated clinic BP protocol with the target of 140 mmHg measured by other clinic BP protocols. Research investigating automated clinic BP measurement protocols is promising[105,106] but future research should establish the exact number of BP readings, as well as the duration of monitoring required to reliably assess BP. At the same time, future research will need to define BP thresholds using automated clinic BP protocols which should be comparable to current international clinic BP protocols in order to enable research translation in clinical practice. However, until reliable clinic BP protocols are established, the assessment of BP and diagnosis of hypertension should ideally be confirmed with out-of-clinic BP measures (i.e. home or 24-hour BP).

The results derived from SPRINT have raised similar questions regarding BP measurement and BP thresholds in children and adolescents.[202] Prehypertension in adolescence is determined by a threshold of BP greater than the 90th percentile of age, height and sex-based criteria or a BP greater or equal to 120/80 mmHg. Although the conclusions and implications of SPRINT are not directly applicable to children and adolescents, the question of which BP threshold to use and how to measure BP (i.e. how many readings should be used?) in this population is still relevant. Chapter 5 examined the direction and magnitude of the changes in BP over consecutive measurements, as well as factors affecting within-visit BPV, in a nationally

representative sample of more than 3,000 children and adolescents from the Australian Health Survey. This is the first descriptive study to quantify very short-term BPV in children and adolescents using a nationally representative sample. The results showed that BPV is highly variable in this population; however, the magnitude of change in BP over consecutive measurement was greater among children and adolescents with higher BP levels, but lower with older age. Moreover, as observed among adults, and in contrast to expectations that BP should always drop over consecutive measurements, systolic BP increased on repeat measurement among a significant proportion of the population. The highly variable BP observed in children and adolescents underscores the need for further research to establish BP thresholds from reliable assessment protocols that take into account this variability. A crucial aspect of clinic BP protocols that remains to be clarified is the number of BP readings required for a reliable assessment. The number of readings used for BP assessment in this population vary from two[140-142] and three BP,[143-146] to four[147] readings. Studies have also used the average of second and third readings discarding the first one,[148] or recorded the lower of three readings.[149] This heterogeneity in the methodology of BP measurement in clinic affects the estimation of hypertension prevalence among children and adolescents and has potentially important implications for the diagnosis of hypertension in clinical practice. Additionally, protocols based on automated, unobserved clinic BP assessment should also be explored in children and adolescents with the aim to establish BP thresholds that are less biased by the white-coat effect. Nevertheless, these results highlight the need for out-of-clinic BP monitoring in order to confirm the diagnosis of elevated BP among children and adolescents.

Chapter 6 represent the first study to examine the effect of novel destiffening interventions (vitamin D supplementation) on long-term BPV, in a patient population that was vitamin D deficient and enriched with vascular risk factors that should have been amenable to benefit from treatment with vitamin D, based on a plethora of cross-sectional studies and small-scale

clinical trials. The results were that vitamin D supplementation was ineffective in lowering long-term BPV or aortic stiffness. The results of Study 6 (which was a sub-study of a multi-cantered trial [VIDEO study] as described in the methodology), seen in the context of the overall results of the VIDEO, show that vitamin D supplementation is ineffective in improving osteoarthritis related outcomes but also in improving cardiovascular health outcomes in an older population with symptomatic knee osteoarthritis and low serum 25-hydroxyvitamin D levels. Moreover, the results of this study are confirmed in study 7 (presented in Appendix 4) which showed that the majority of randomized controlled trials exploring the effect of vitamin D supplementation on cardiovascular health outcomes are ineffective, despite the sample size, vitamin D dose and the absence or presence of vitamin D deficiency. Further evidence supporting our results on the lack of efficacy of vitamin D supplementation has been recently published from the first large-scale (n=5,108), high-dose randomized controlled trial (100 000 IU of vitamin D; median follow-up 3.3 years) with *a priori* outcomes of incident cardiovascular disease and death (secondary outcomes were myocardial infarction, angina, heart failure, hypertension, arrhythmias, arteriosclerosis, stroke, and venous thrombosis).[203] Nevertheless, a definite answer as to whether vitamin D supplementation could improve cardiovascular health will be provided in the near future as several large-scale trials are ongoing.[204]

Appendix 1

Supplementary material for Chapter 1

Table 1. Data chart

Supplementary table 1. Data chart

Short-term BPV (using 24-hour BP monitoring over hours)							
Author	**Year**	**Type of BPV**	**BPV (mmHg)**	**Age (years)**	**SBP (mmHg)**	**Readings**	**Duration (hours)**
Saito, et al.[205]	2016	Awake	13.1	68.8	120	NC	Used diaries
Ruan, et al.[206]	2016	Day	11.3	61.4	125.9	3/hour	16
McDonald, et al.[207]	2016	Day	11.2	70	131.35	2/hour	10
Juhanoja, et al.[208]	2016	Day	13.6	54	138.4	4/hour	12
Boardman, et al.[209]	2016	Day	15	31	122.1	2/hour	16
Wu, et al.[210]	2016	Day	11.8	57.7	125.7	2/hour	16
Kang, et al.[211]	2016	Day	15.4	52.4	138.6	NC	16
Miroslawska, et al.[212]	2015	Day	23	53	157	3/hour	15
Madden, et al.[213]	2015	Awake	12.8	60.2	131.4	NC	Used diaries
Karpov, et al.[214]	2015	Day	9.3	52.7	155	4/hour	16
Johansson, et al.[215]	2015	Day	13.5	47.8	NR	4/hour	17
Symonides, et al.[216]	2014	Day	12	45.4	130	4/hour	17
Stabouli, et al.[217]	2014	Day	11.3	12	119.5	NC	14
Tatasciore, et al.[218]	2013	Day	12.7	54.9	138.1	4/hour	16
Leoncini, et al.[219]	2013	Day	18.5	47.1	146	4/hour	16
Ciobanu, et al.[220]	2013	Day	17	57.4	148	NC	14
Zuern, et al.[221]	2012	Day	15.1	68.9	155	3/hour	12
Cahan, et al.[222]	2012	Day	14	55	141	3/hour	17
Kotsis, et al.[223]	2011	Day	12.5	21.3	116.8	NC	14
Eguchi, et al.[224]	2009	Day	18	67.8	145	NC	NR
Masuda, et al.[225]	2009	Awake	13.3	61.2	152	NC	Used diaries
Verdecchia, et al.[226]	2007	Day	13.1	51.2	142	4/hour	12

Tatasciore, et al.[72]	2007	Day	13	53.2	139.9	4/hour	16
Shintani, et al.[227]	2007	Awake	15.3	66.2	130.5	NC	Used diaries
Ichihara, et al.[228]	2006	Day	19	53.3	153	2/hour	16
Abramson, et al.[229]	2006	Day	10.2	43.3	117.4	2/hour	16
Mid-term BPV (using home BP monitoring over days)							
Author	**Year**	**Type of BPV**	**BPV (mmHg)**	**Age (years)**	**SBP (mmHg)**	**Readings**	**Duration (Days)**
Okada, et al.[230]	2014	Morning	7.4	70	135.4	1	7
		Evening	8.5	70	129.5	1	7
Liu, et al.[231]	2015	Day-to-Day	14.7	84.35	146.25	3	7
Fukui, et al.[6]	2013	Morning	10.2	65.9	NR	3	14
		Evening	10.7	65.9	NR	3	14
Hashimoto, et al.[232]	2012	Morning	8.4	58.6	127.8	1	28
Hoshide, et al.[233]	2012	Day-to-Day	12.9	65.7	145.9	3	7
Okada, et al.[234]	2012	Morning	7.2	66	136	1	7
		Evening	7.9	66	130.2	1	7
Kikuya, et al.[235]	2008	Morning	8.6	59.4	124.6	1	26
Ishikura, et al.[236]	2012	Morning	7.9	67	135.8	1	14
Kato, et al.[237]	2010	Morning	8.6	62	123.4	1	28
		Evening	8.8	62	121	1	28
Satoh, et al.[7]	2015	Morning	8.3	61.9	121	1	28
Shin, et al.[238]	2016	Morning	7.9	56.2	138.8	1	6
		Evening	8.4	56.2	137.57	2	6
Juhanoja, et al.[208]	2016	Day-to-Day	5.7	54	130	1	7
Karpov, et al.[214]	2015	Morning	7.7	52.7	148.4	1	6
		Evening	7.5	52.7	147	1	6

		Day-to-Day	7.6	52.7	NR	2	6
Johansson, et al.[239]	2010	Day-to-Day	6.4	56.4	129.7	3	7
Johansson, et al.[215]	2015	Day-to-Day	4.5	47.8	118.1	1	7
		Morning	6	47.8	NR	1	7
		Evening	6.2	47.8	NR	1	7
Long-term BPV (using clinic BP monitoring over visits)							
Author	**Year**	**Type of BPV**	**BPV (mmHg)**	**Age (years)**	**SBP (mmHg)**	**Visits**	**Duration (years)**
Chia, et al.[9]	2016	Visit-to-visit	14.2	55.5	136.7	3 to 4 /year	15
Tedla, et al.[240]	2016	Visit-to-visit	8.7	57	113.5	3 to 5	9.5
Sohn, et al.[8]	2016	Visit-to-visit	12.7	53.7	134.5	6	3.5
Wang, et al.[241]	2016	Visit-to-visit	10.9	48	128.1	1 visit every 2 years	6
Faramawi, et al.[242]	2016	Visit-to-visit	5.8	37	NR	2	NR
Wang, et al.[243]	2016	Visit-to-visit	10.93	51	NR	4	4
Nagai, et al.[244]	2016	Visit-to-visit	32.5	79.7	145.7	12	1
Ogliari, et al.[245]	2016	Visit-to-visit	16.74	75.2	153.7	7	1.5
Yano, et al.[246]	2016	Visit-to-visit	7.8	44	126.8	3	0.42
Takao, et al.[247]	2015	Visit-to-visit	10.7	55.7	129.1	9 (median)	1
Rossignol, et al.[248]	2015	Visit-to-visit	11.05	64	126.3	12	6.8
Gondo, et al.[249]	2015	Visit-to-visit	10.2	69	129	4	0.5
Darabont[250]	2015	Visit-to-visit	6.16	47.14	132.37	2	NR
Jo, et al.[251]	2015	Visit-to-visit	12.9	46.9	130.3	>12 visits	1.66
Nakano, et al.[252]	2015	Visit-to-visit	14.3	62.7	128.6	10	NR
Chang, et al.[253]	2015	Visit-to-visit	11.9	NR	NR	2 to 5	NR
Okada, et al.[230]	2014	Visit-to-visit	13.4	70	129.2	10	2
Lau, et al.[254]	2014	Visit-to-visit	16	70	142	12	6.5

Yokota, et al.[255]	2014	Visit-to-visit	18.3	67	151	12	2.7
Lau, et al.[256]	2014	Visit-to-visit	14	66	134	18	6.75
Faramawi, et al.[257]	2014	Visit-to-visit	6.82	43	NR	2	NR
Faramawi, et al.[258]	2014	Visit-to-visit	6.79	42	NR	2	NR
Cao, et al.[259]	2014	Visit-to-visit	10.35	50.44	126.99	3	6
Noshad, et al.[260]	2014	Visit-to-visit	9.86	52	122.04	NR	2.6
McMullan, et al.[261]	2014	Visit-to-visit	12	59.5	146.2	4	0.25 - 1
Song, et al.[262]	2014	Visit-to-visit	17.36	54	156.5	4	1
Yano, et al.[263]	2014	Visit-to-visit	8.7	25	118.8	8	25
Yu, et al.[264]	2014	Visit-to-visit	8.87	64	NR	at least 6	4
Masugata, et al.[265]	2014	Visit-to-visit	10	73	130	NR	1
Lattanzi, et al.[266]	2014	Visit-to-visit	8.33	76	137.16	5	1
Selvarajah, et al.[267]	2014	Visit-to-visit	17.4	66	144	24	0.25
Lau, et al.[268]	2014	Visit-to-visit	14	68	131	18	NR
Chowdhury, et al.[269]	2014	Visit-to-visit	13	48.3	168	8 (median)	4.1
Poortvliet, et al.[270]	2014	Visit-to-visit	14.4	75.2	153.4	9	3.2
Yokota, et al.[29]	2013	Visit-to-visit	15.3	69.5	140	12	NR
Sabayan, et al.[271]	2013	Visit-to-visit	14.8	75.3	153.1	every 3 months	3.2
Obara, et al.[272]	2013	Visit-to-visit	6.9	66.1	137.8	2	0.08
Mallamaci, et al.[273]	2013	Visit-to-visit	11	64	134	2 to 7	3
Muntner, et al.[274]	2013	Visit-to-visit	6.3	47.9	115.2	6	0.06
Hata, et al.[275]	2013	Visit-to-visit	11	66	138	6	2.4
Levitan, et al.[31]	2013	Visit-to-visit	6.8	48	133.5	7	4
Kawai, et al.[276]	2013	Visit-to-visit	11.42	61.7	138.6	6	0.5 - 1
Epstein, et al.[277]	2013	Visit-to-visit	11.4	75.2	133.1	up to 7 visits	3

Suchy-Dicey, et al.[278]	2013	Visit-to-visit	9	72	NR	5	5
Rossignol, et al.[279]	2012	Visit-to-visit	14.82	66	142.7	17	2
Eguchi, et al.[280]	2012	Visit-to-visit	13.7	67	154	37	5.6
Poortvliet, et al.[281]	2012	Visit-to-visit	14.1	75.2	153.7	5	1
Levitan, et al.[28]	2012	Visit-to-visit	5.6	48.9	NR	3	1.5
		Visit-to-visit	6.8	48.9	NR	7	1.5
		Visit-to-visit	7.5	48.9	NR	18	1.5
Mancia, et al.[48]	2012	Visit-to-visit	8.6	56	140.9	at least 7	4
Shimbo, et al.[282]	2012	Visit-to-visit	10.9	NR	125.8	8	5.4
Muntner, et al.[283]	2011	Visit-to-visit	13.5	75	135.2	7	2.8 (median)
		Visit-to-visit	12.2	75	135.4	4	NR
Nagai, et al.[284]	2011	Visit-to-visit	32.4	79.9	145.6	12	1
Masugata, et al.[285]	2011	Visit-to-visit	11	69	129.12	every 1 or 2 months	1
Muntner, et al.[286]	2011	Visit-to-visit	7.7	NR	NR	3	NR
Yeh, et al.[287]	2016	Visit-to-visit	14.8	63.4	136.8	45	10

NC=could not be calculated; NR = not reported; BPV = blood pressure variability (calculated as standard deviation); SBP = systolic BPV.

Appendix 2

Supplementary material for Chapter 4

Survey design and ethics approvals

The AHS used a random multistage sampling strategy, details of which has been previously published.[96] All the data used in this analysis were provided by the ABS through Confidentialised Unit Record Files (CURFs) which were accessible via the Remote Access Data Laboratory (RADL) in order to monitor the amount and information of data being released and restrict information that may enable identification of individuals (i.e. instead of reporting home of business address, CURFs report the State).[288]

Approximately 9,000 participants had blood and urine tests completed for a range of biomedical markers. Ethics approval for the National Health Measures Survey, which included the physical measures and biomedical tests, were provided by the Australian Government Department of Health and Ageing's Departmental Ethics Committee.[289] Participants were provided written information on the survey and signed informed consent.

Anthropometry

Height and weight were measured and body mass index (BMI; kg/m^2) calculated as the weight (kg) divided by the height^2 (m^2). For validation purposes, 10% of the participants were randomly selected for a second height measurement and if there was a difference greater than one centimetre then a third reading was taken.[133] Waist circumference (cm) was also measured according to guidelines.[134]

Biomedical measures

Participants were referred to a collection clinic for blood and urine tests, or if unable to attend a collection clinic, provided samples at home environment. All samples were analysed at a central laboratory.[289] For plasma glucose, low-density lipoprotein cholesterol and triglycerides, samples were provided after a fasting period of 8 hours. The blood and urine samples were

analysed to provide risk information on cardiovascular disease, diabetes, kidney disease and liver function.[290]

Secondary analyses

The interaction between age and SBP1 remained significant after adjusting for age, sex, body mass index and other cardiovascular risk factors (cardiovascular disease status, smoking status, cholesterol levels; $p<0.0001$). As there was a very strong correlation between PP and SBP we did not adjust for the level of PP to avoid multicollinearity issues. Cardiovascular disease included conditions that could affect ΔSBP or secondary outcomes:

1. Diabetes (including type I, type II or having high blood/urine sugar levels)
2. High cholesterol levels
3. Hypertension
4. Heart disease (ischaemic heart disease, heart failure, other heart diseases)
5. Tachycardia
6. Stroke
7. Other cerebrovascular diseases
8. Diseases of arteries, arterioles, and capillaries
9. Low BP
10. Kidney disease

Results pertaining to the interaction between SBP level and age were unchanged when analysis was confined to the differences between the SBP2 and SBP3 or, when analysis took into

account the total variation within three consecutive measurements as reflected by the coefficient of variation (SBP CV; [standard deviation/mean]*100) (Figure 3). Analyses were also conducted using the DBP instead of the SBP readings; however, as compared to the SBP, there were no age- or BP-related patterns, nor were similar relationships observed in relation to the DBP difference between measurements. Testing the effect of age and the level of first PP (PP1) on the difference between PP1 and PP2 revealed a similar interaction as with age and SBP1 ($p<0.0001$); however, it did not remain significant when the difference between PP1 and PP2 was plotted against the average level of PP1 and PP2 ($p=0.11$).

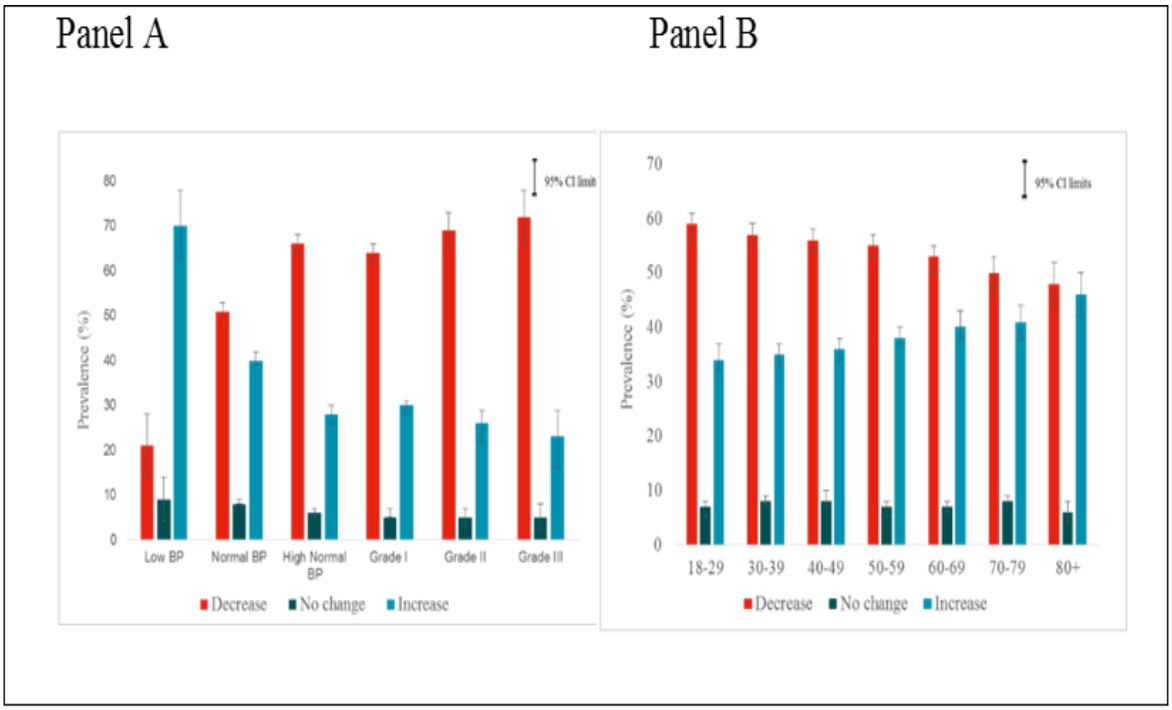

Figure 1. The direction of change in SBP from first to second SBP readings across BP categories classified from the first SBP readings (Panel A) and across age groups (Panel B). SBP was classified as following; normal (90-129 mmHg), high normal (130-139 mmHg), grade I (140-159 mmHg), grade II (160-179 mmHg) and grade III (≥180 mmHg). Low BP was defined as SBP <90 mmHg.

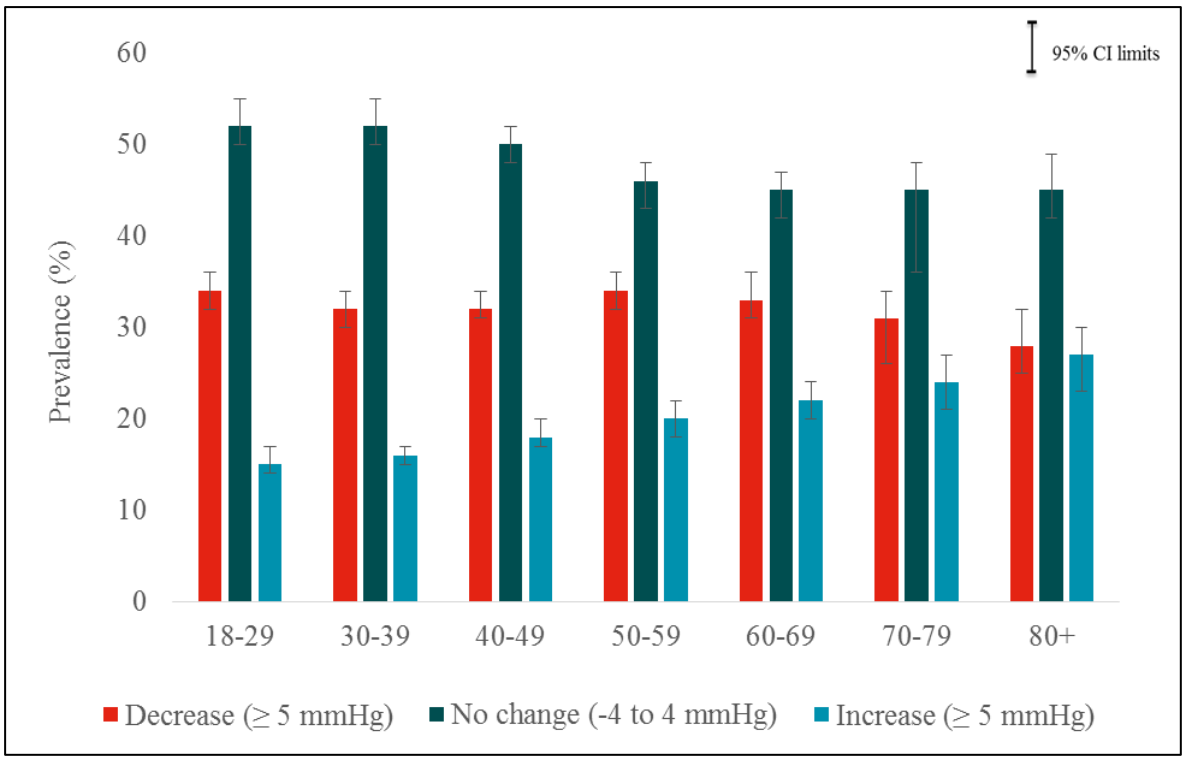

Figure 2. The direction of change in SBP from first to SBP readings across age groups (Panel B).

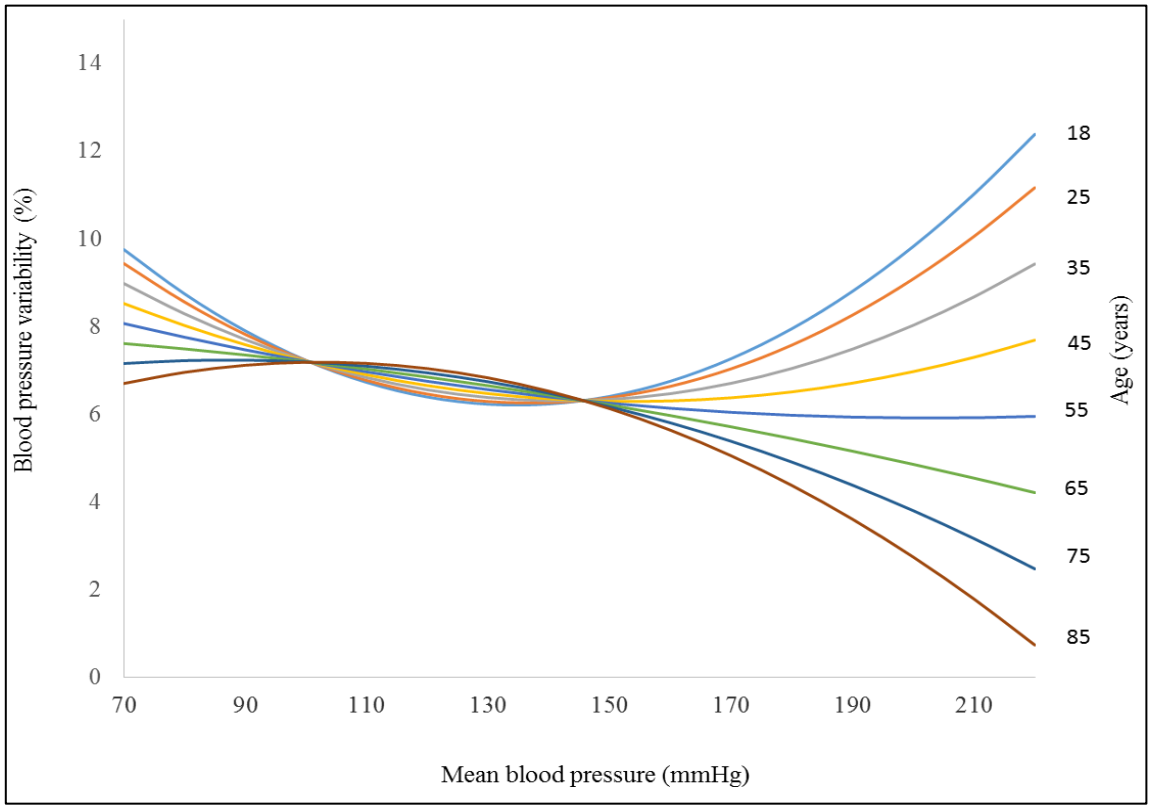

Figure 3. Modification effect of age on the relationship between the variability in systolic blood pressure across three readings and the level of mean systolic blood pressure as defined by the average of first, second and third readings.

Appendix 3

Supplementary material for Chapter 5

Survey design and ethics approvals

The Australian Health Survey used a random multistage sampling strategy, details of which has been previously published.[96] All the data used in this analysis were provided by the ABS through Confidentialised Unit Record Files (CURFs) which were accessible via the Remote Access Data Laboratory (RADL) in order to monitor the amount and information of data being released and restrict information that may enable identification of individuals (i.e. instead of reporting home of business address, CURFs report the State).[288]

Weighting

The analysis utilised person-weights[96] provided by the ABS, which ensured that any disproportionate sampling of certain groups was taken into account. Replicate weights provided by the ABS were used to calculate standard errors and 95% confidence intervals using the Jackknife delete-1 method.

Appendix 4

Effectiveness of Vitamin D Supplementation for Cardiovascular Health Outcomes

Published link:

https://www.ncbi.nlm.nih.gov/pubmed/28229054

Veloudi P., Jones G., Sharman JE Effectiveness of Vitamin D Supplementation for Cardiovascular Health Outcomes. Pulse (Basel). 2017;4(4):193-207.

doi: 10.1159/000452742

Abstract

There is a plausible physiological theory, supported by many observational studies, that vitamin D supplementation should be effective for improving cardiovascular end points, such as blood pressure (BP), large artery stiffness, atherosclerosis, endothelial function and clinical events. However, results from randomised controlled trials (RCTs) have been inconsistent. In this review, we evaluated the evidence regarding the effectiveness of vitamin D supplementation for cardiovascular surrogate and hard clinical end points. RCTs were assessed in terms of sample size, duration of supplementation, baseline vitamin D level inclusion criteria (i.e., absence of vitamin D deficiency), dosage of vitamin D and population under investigation. Forty-five RCTs were identified. Eight RCTs with BP and 6 RCTs with large artery stiffness as the end points were found to comply with guidelines for the optimal design of clinical trials evaluating nutrient effects. Only 2 of the RCTs with an optimal design were effective in decreasing BP with vitamin D supplementation, although these were of moderate sample size (<150) and very short duration (8 weeks for both), whilst no RCT was effective in reducing large artery stiffness. Similar results were observed for atherosclerotic and endothelial function markers as end points. Only 1 RCT reported cardiovascular events as an end point and found neither increased nor decreased incident cardiovascular events over 7 years of follow-up. In conclusion, results from published RCTs indicate that vitamin D supplementation is ineffective in improving cardiovascular health among various patient populations, including in the presence or absence of vitamin D deficiency.

Introduction

Vitamin D (VitD), a lipid-soluble vitamin, plays a well-recognised role in musculoskeletal health,[291,292] but evidence also suggests a critical role in blood pressure (BP) regulation and vascular health.[293] In vivo and in vitro studies have suggested a number of pathways by which VitD could directly benefit the vasculature[159,294,295] in addition to acting as a negative regulator of the renin-angiotensin system to influence BP control[160,296] and modify large artery stiffness.[53] Observational data during the last decade suggest a relationship of low VitD levels with cardiovascular end points, including coronary artery disease, myocardial infarction (MI), heart failure (HF), stroke and cardiovascular death. Additionally, more than 4 decades of cross-sectional research generally show a consistent inverse association between VitD levels and surrogate cardiovascular markers of BP, large artery stiffness, atherosclerotic burden and endothelial function. Nevertheless, the highest level of evidence, derived from well-designed randomised controlled trials (RCTs), has been inconsistent as to whether VitD exerts cardioprotective effects. In this short narrative review, we sought to summarise the observational data and critique evidence from published RCTs on the effect of VitD supplementation on cardiovascular surrogate and hard clinical end points, with particular consideration of study design (i.e., sample size, duration of supplementation, selection of VitD-deficient subjects, VitD dose and population under investigation).

Summary of Observational Data on the Relationship between Serum VitD and Cardiovascular Disease Outcomes

Cardiovascular Events and Cardiovascular Death

Studies examining whether VitD is associated with cardiovascular events or cardiovascular death are seen in Figure 1. VitD deficiency has been associated with an increased risk of death, HF, MI or stroke in healthy postmenopausal women,[297] as well as with an increased risk of

sudden cardiac death or fatal or non-fatal stroke, non-fatal MI and death related to other heart diseases among diabetic patients with chronic kidney disease (CKD).[298] In a small study of patients with acute coronary syndrome, VitD deficiency was also independently associated with in-hospital cardiovascular death.[299] In general population studies, low VitD levels were associated with an increased incidence of coronary artery disease, MI, HF, stroke and all-cause death,[300] as well as increased cardiovascular death.[301] Several other studies have demonstrated an increased risk of cardiovascular death associated with low VitD levels among different patient groups including HF outpatients (in a small study),[302] patients with metabolic syndrome and cardiovascular symptoms,[303] and patients with chronic obstructive pulmonary disease.[304] Furthermore, data extracted from medical records of 126 men with moderate CKD and VitD deficiency showed that VitD treatment was associated with decreased cardiovascular events.[305] In this study, the treatment group was defined based on an increase in serum VitD levels by 25% from baseline within 6 months, whilst the remaining patients were considered as controls. The risk of cardiovascular death was also lower among haemodialysis patients regularly using VitD supplements in a Japanese hospital (the VitD dose varied according to prescription).[306] Overall, these data suggest fairly consistently that low VitD levels are associated with an increased risk of cardiovascular events, including death; in addition, the data hint towards the potential for VitD supplementation possibly reversing the adverse cardiovascular effects of low VitD.

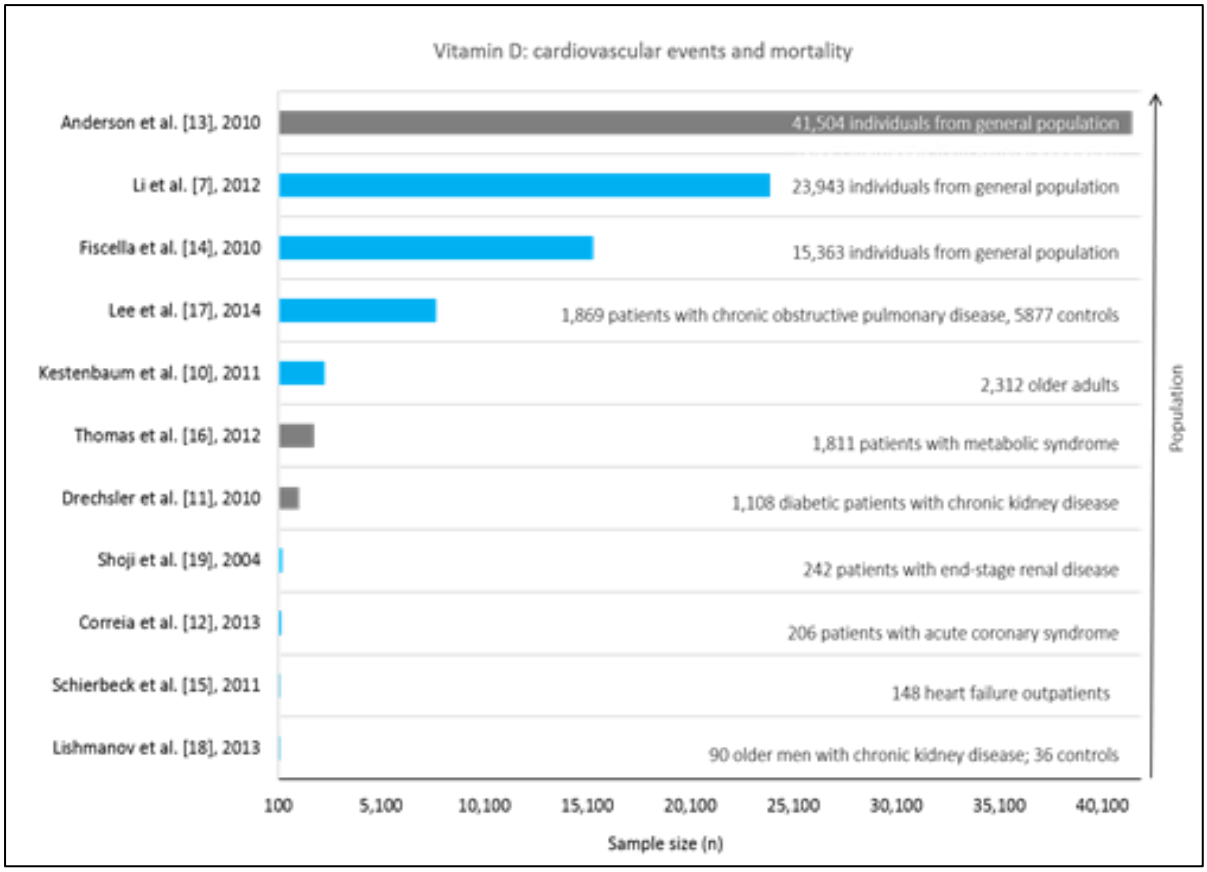

Figure 1. Longitudinal cohort and case-control studies on the association between vitamin D and incidence of cardiovascular events or cardiovascular mortality according to sample size and population characteristics.
Blue bars indicate studies that reported significant associations between vitamin D and cardiovascular end points, and grey bars indicate studies that reported no significant associations.

BP and Large Artery Stiffness

Brachial BP, large artery stiffness (as measured by carotid-to-femoral pulse wave velocity [cfPWV], an estimate of aortic pulse wave velocity and the current "gold standard" measure of large artery stiffness)[57] and central hemodynamic parameters (such as augmentation index [AIx], a marker of central systolic loading) are independent predictors of cardiovascular risk.[153,154] Many observational studies have reported an inverse association between low VitD levels and brachial BP in large samples from the general population,[157,307,308] but also among Peruvian adolescents,[309] middle-aged individuals,[310] people aged >60 years and the elderly,[311,312] pregnant women and women aged 20--80 years.[313,314] Similarly, the evidence

from medium to large observational studies shows strong and independent inverse associations between serum VitD and cfPWV among diverse study populations.[156,315-320] Other markers of regional arterial stiffness, including increased carotid-radial pulse wave velocity and brachial-ankle pulse wave velocity, have been associated with lower VitD levels among people with type 2 diabetes mellitus (T2DM).[321,322] An inverse association between VitD levels and AIx has also been shown in pre-diabetic individuals and cardiac or kidney clinic outpatients.[323,324] In summary, observational evidence is consistently and strongly supportive of an association between VitD, BP and vascular health.

Markers of Atherosclerotic Burden and Endothelial Function

Strong relationships have also been observed between VitD level, atherosclerotic burden and endothelial function markers. Carotid intima medial thickness (CIMT), a marker of large artery atherosclerosis, known to be predictive of cardiovascular events,[325] was found to be inversely associated with low VitD levels among different populations, including apparently healthy, predominantly white older individuals,[326] relatively healthy Chinese women[327] and patients with peripheral arterial disease.[328] Additionally, endothelial flow-mediated dilation (FMD; the change in vessel diameter during reactive hyperaemia after cuff release), a marker of nitric oxide-mediated endothelial function, has been shown to be decreased in the presence of low serum VitD levels in patients with T2DM,[329] end-stage renal disease[330] and CKD.[331] Altogether, the above observational evidence points to the need for intervention trials to determine if there is a causative link between low VitD and poor cardiovascular health.

Summary of RCT

Data on the Effect of VitD Supplementation on Cardiovascular Diseases Outcomes

Cardiovascular Events and Cardiovascular Death, BP and Large Artery Stiffness

A total of 45 RCTs on the effect of VitD supplementation on cardiovascular surrogate and hard clinical end points were identified through an English language search of PubMed and Google. To our knowledge, only 1 RCT has investigated the effects of VitD supplementation on hard clinical end points, which included incident MI, stroke and death related to coronary heart disease. This study randomised 36,282 postmenopausal women to 200 IU VitD plus calcium carbonate twice daily or placebo for 7 years and found that VitD supplementation did not improve cardiovascular risk.[332] Importantly, this study was criticised for using a low VitD dose and not measuring serum VitD levels.[333,334] In terms of effects on BP, some RCTs have reported significant improvements in brachial BP after short-term supplementation of VitD among participants encompassing a variety of clinical characteristics, including patients with hypercalcaemia[335] or impaired glucose tolerance,[173] patients with primary hyperparathyroidism,[336] patients with T2DM,[171,337,338] patients with elevated BP and VitD deficiency[339] and women with VitD deficiency.[165] However, the majority of RCTs have been ineffective in improving brachial BP in a range of populations as shown in Table 1.[161-163,166,168,174,176,340-351] Indeed, a recent individual-level meta-analysis of RCTs concluded that VitD was ineffective in lowering BP.[352]

Table A4.1 Effect of vitamin D supplementation on cardiovascular outcomes in randomised controlled trials in various populations

Study [ref.], year	Population (group *n*)	Vitamin D level; inclusion criteria, ng/mL	Effect on brachial BP†	Effect on cBP†	Effect on cfPWV†	Effect on AIx†
Dalan et al. [63], 2016	Individuals with T2DM and hypovitaminosis D (treatment = 33, control = 31)	<30	Null	–	–	Increase
Zaleski et al. [85], 2015	Individuals with pre-hypertension and vitamin D deficiency (low dose = 22, high dose = 19)	≤25*	Null	–	Null	Decrease
Veloudi et al. [77], 2015	Older individuals with osteoarthritis and vitamin D deficiency (intervention = 118, control = 123)	<24*	Null	Null	Null	Null
Pilz et al. [92], 2015	Individuals with hypertension (intervention = 100, control = 100)	<30	Null	–		
McGreevey et al. [84], 2015	Individuals with vitamin D deficiency (low dose = 60, high dose = 50)	<20*	–		Null	Decrease
Gepner et al. [62], 2015	Healthy postmenopausal Native American women (low dose = 49, high dose = 49)	<60	Null	Null	–	Null
Thethi et al. [90], 2015	Individuals with T2DM and stage 3 or 4 CKD (treatment = 30, control = 30)	No restrictions	Null	–	–	–
Arora et al. [94], 2015	Individuals with pre-hypertension or stage 1 hypertension and low vitamin status (low dose = 188, high dose = 175)	≤25*	Null	–	–	–
Witham et al. [95], 2014	Patients aged >70 years with orthostatic hypotension (intervention = 38, control = 37)	<30	Null	–	–	–
Witham et al. [116], 2014	Patients with resistant hypertension (intervention = 31, control = 30)	<30	Null	–	–	–
Scragg et al. [68], 2014	Healthy adults (intervention = 161, control = 161)	No restrictions	Null	–	–	–
Ryu et al. [64], 2014	Individuals with T2DM (intervention = 40, control = 41)	<20*	Null	–	Null	Null
Nasri et al. [58], 2014	Individuals with T2DM (intervention = 30, control = 30)	No restrictions	Decrease		–	–

Mozaffari-Khosravi et al. [59], 2015	Patients with elevated BP and vitamin D deficiency (intervention = 19, control = 20)	<30	Decrease	–	–	–
Martins et al. [75], 2014	Overweight and obese African Americans with elevated BP (intervention = 65, control = 65)	10–25*	–	–	–	Decrease
Dreyer et al. [70], 2014	Patients with CKD (intervention = 20, control = 18)	<16*	Null	–	Null	–
Yiu et al. [65], 2013	Individuals with T2DM (intervention = 50, control = 50)	<30	Null	–	–	–
Witham et al. [96], 2013	Older patients with isolated hypertension (intervention = 80, control = 79)	<30	Null	–	–	–
Witham et al. [73], 2013	Patients with a history of myocardial infarction (intervention = 39, control = 36)	No restrictions	Null	–	–	–
Forman et al. [79], 2013	Black individuals (intervention 1,000 IU/day = 68, intervention 2,000 IU/day = 73, intervention 4,000 IU/day = 70, control = 72)	No restrictions	Decrease‡	–	–	–
Breslavsky et al. [66], 2013	Individuals with T2DM (intervention = 24, control = 23)	No restrictions	Null	–	–	Decrease
Marckmann et al. [71], 2012	CKD patients with hypovitaminosis D (intervention = 25, control = 24)	<20*	Null	–	Null	Null
Stricker et al. [72], 2012	Patients with peripheral arterial disease (intervention = 31, control = 31)	<30	–	–	–	Null
Witham et al. [69], 2012	Stroke patients with well-controlled BP (intervention = 30, control = 28)	<30	Null	–	–	–
Gepner et al. [61], 2012	Postmenopausal women (intervention = 55, control = 54)	<60	Null	Null	Null	Null
Larsen et al. [83], 2012	Individuals with hypertension (intervention = 55, control = 57)	No restrictions	Null	Decrease	Null	Null
Witham et al. [57], 2010	Individuals with T2DM (intervention 100,000 IU = 19, intervention 200,000 IU = 20, control = 22)	<40	Decrease	–	–	–
Jorde et al. [76], 2010	Overweight and obese subjects (intervention 40,000 IU = 150, intervention 10,000 IU = 139, control = 149)	No restrictions	Increase	–	–	–

Dong et al. [74], 2010	Black youth (intervention = 23, control = 21)	No restrictions	Null	–	Decrease	–
Daly et al. [78], 2009	Men aged >50 years (intervention = 73, control = 67)	No restrictions	Null	–	–	–
Zittermann et al. [97], 2009	Healthy overweight subjects with inadequate vitamin D status (intervention = 82, control = 83)	No restrictions	Null	–	–	–
Sugden et al. [56], 2008	Individuals with T2DM and low vitamin D levels (intervention = 17, control = 17)	<20*	Decrease	–	–	–
Margolis et al. [93], 2008	Postmenopausal women (intervention = 15,176, control = 18,106)	No restrictions	Null	–	–	–
Pfeifer et al. [60], 2001	Women with vitamin D deficiency (intervention = 74, control = 74)	<20*	Decrease	–	–	–
Scragg et al. [67], 1995	Healthy adults (intervention = 95, control = 94)	No restrictions	Null	–	–	–
Pan et al. [117], 1993	Elderly individuals (intervention Ca = 14, intervention vitamin D = 14, intervention Ca + vitamin D = 15, control = 15)	No restrictions	Null	–	–	–
Orwoll et al. [98], 1990	Normotensive men (intervention = 35, control = 30)	No restrictions	Null	–	–	–
Lind et al. [118], 1989	Patients with essential hypertension (n = 39, allocation not reported)	No restrictions	Null	–	–	–
Lind et al. [55], 1988	Patients with primary hyperparathyroidism (intervention = 15, control = 16)	No restrictions	Decrease	–	–	–
Lind et al. [54], 1988	Men with impaired glucose tolerance (intervention = 33, control = 32)	No restrictions	Decrease	–	–	–
Lind et al. [53], 1987	Patients with marginal, intermittent hypercalcaemia (intervention = 29, control = 57)	No restrictions	Decrease	–	–	–

cBP = central blood pressure; cfPWV = carotid-to-femoral pulse wave velocity; AIx = augmentation index; T2DM = type II diabetes mellitus. *Vitamin D deficiency (<25 ng/ml). †Statistically significant increase or decrease, or no change (null) with vitamin D supplementation.

‡No longer statistically significant after adjustment for BP differences at baseline. This article is not an all-inclusive systematic review and results may not reflect all trials investigating the outcomes of interest.

Organ damage is more closely associated with central BP (cBP) than brachial BP[353] and, whilst individuals could have similar brachial systolic BP levels, central systolic BP may significantly differ.[354] Only 1 study has shown VitD supplementation to be effective in decreasing cBP (Table 1),[355] whereas others have shown no improvement in older individuals with osteoarthritis[350] or among postmenopausal women.[162,340] VitD supplementation was also ineffective in changing visit-to-visit BP variability.[350] Central arterial stiffness is strongly associated with BP,[53] and there is a need for interventions aimed at improving central arterial stiffness independent of BP changes. Virtually all RCTs investigating the effect of VitD supplementation on cfPWV reported no improvement[162,342,346,347,350,355-357] apart from 1 study in black youths.[161] The results from RCTs on AIx are more contradicting; no improvement was reported in the majority of studies.[162,176,340,342,347,350,355] Alternatively, decreases in AIx were observed among patients with T2DM,[344] overweight people with elevated BP,[163] people with VitD deficiency[356] and those with pre-hypertension and VitD deficiency.[357] Conversely, 1 study reported significant increases in AIx in patients with T2DM and hypovitaminosis.[341] No improvements were reported in a small number of RCTs for carotid-radial PWV and brachial-ankle PWV.[161,163,342,343] In summary, the majority of evidence from RCTs is not supportive of VitD treatment for improving BP control or large artery stiffness.

Markers of Atherosclerotic Burden and Endothelial Function

RCTs examining markers related to atherosclerotic burden as measured by CIMT are limited. No improvement in CIMT was reported among postmenopausal women; however, in this trial the supplementation regimen contained both VitD and vitamin K.[358] In terms of brachial FMD, improvements were reported among African Americans[359] and patients with T2DM,[337] whilst brachial FMD was improved after 8 weeks in stroke patients, but this did not persist at 16 weeks.[345] No improvements in brachial FMD were observed among patients with coronary artery disease,[360] patients with T2DM,[338,343] HIV-infected individuals[361] and postmenopausal

women.[162,362] Lastly, VitD was found to have no effect on the reactive hyphaemia index in patients with T2DM or after MI.[341,348] Based on current evidence, VitD supplementation appears to be ineffective in improving CIMT or FMD.

Critical Factors to Consider Regarding the Interpretation of RCT Data

Adequate Sample Size

Sample size should be adequate to determine a clinically relevant change from intervention, and this will vary depending on outcomes. With respect to BP, we have calculated that at least 100 participants in each randomisation arm are needed to detect at least 4.5 mm Hg between-group change in brachial BP or cBP.[363] Figure 2 depicts RCTs in relation to their sample size and effectiveness of VitD treatment for cardiovascular end points. Generally, most of the effective trials were of a smaller sample size. Seven RCTs with an apparently appropriate sample size have investigated the effects of VitD on brachial BP, and from these, 6 reported no significant improvement.[169,174,349,350,364,365] Only 1 trial reported significant BP lowering, and this was in a population of black individuals;[166] importantly though, this effect was no longer significant after correcting for between-group differences in BP at baseline. We recently published the largest RCT investigating changes in cBP, cfPWV and AIx, which found no significant effects of VitD (Figure 2).[350] Overall, Figure 2 shows that in the larger RCTs with a sample size >200, VitD has been ineffective in improving cardiovascular surrogate markers.

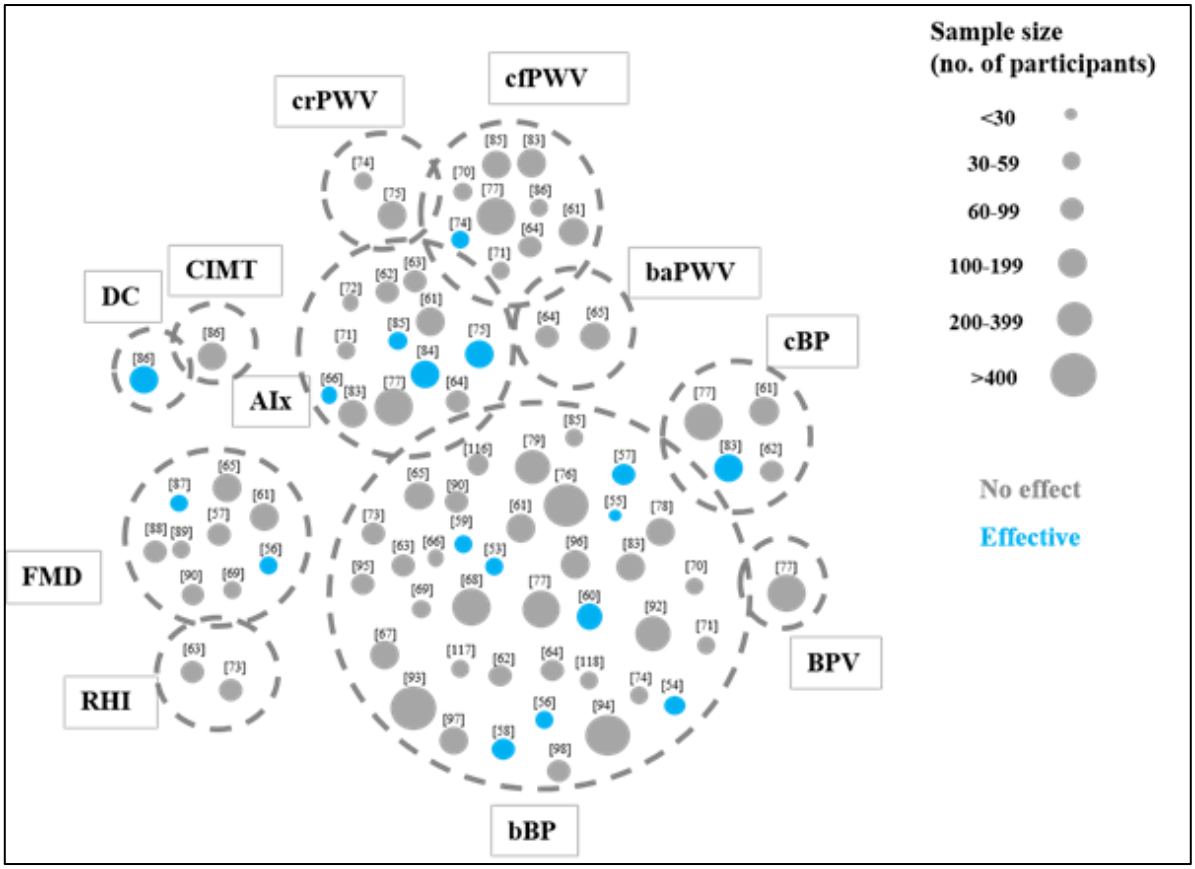

Figure 2. Randomised controlled trials (RCTs) with vitamin D supplementation as the intervention according to sample size and the outcome in terms of effective or no effect on cardiovascular endpoints (reported either as primary or secondary outcomes).
Each grey dotted circle represents a different cardiovascular end-point and the size of the circle is proportional to the number of RCTs for each end-point. Blue circles indicate a significant improvement with vitamin D supplementation (effective RCTs) and grey circles indicate RCTs had no significant effect on respective endpoints. Numbers above the circles represent the corresponding RCT reference number. bBP = brachial blood pressure; RHI = reactive hyperaemia index; FMD = flow-mediated dilation; DC = distensibility coefficient of the carotid artery; CIMT = carotid intimal medial thickness; AIx = augmentation index; crPWV = carotid-radial pulse wave velocity; cfPWV = carotid-femoral pulse wave velocity; baPWV = brachial-ankle pulse wave velocity; cBP = central blood pressure; BPV = visit-to-visit blood pressure variability. This article is not an all-inclusive systematic review and results may not reflect all trials investigating the outcomes of interest.

Adequate Length of Intervention Period

The generalizability of RCT findings must be interpreted in the context of the duration of intervention, given that a short-term RCT may produce results discordant with long-term effects. Although there is no consensus as to the minimum duration of VitD intervention for maximum improvements in BP, interventions of <6 months may not be adequate to modify the

structural characteristics of the large arteries (i.e., improving arterial stiffness independent of BP). The majority of the RCTs included in this review had a duration of ≤6 months (76%), whilst approximately half of these short-term RCTs had a duration of ≤3 months. Figure 3 shows RCTs according to trial duration and effect of estimated monthly dose on brachial BP and cfPWV. Only 10 RCTs examining the effects of treatment on brachial BP had a duration ≥12 months. Importantly, none of these longer-term RCTs observed an improvement in brachial BP with VitD supplementation.[174,175,344,349-351,366-368] Additionally, the only longer-term (1 year) RCT investigating the effects of VitD on cfPWV was ineffectual.[350] Although Breslavsky et al.,[344] showed an improvement in AIx after 1 year of supplementation among patients with T2DM, this was a small study (*n* = 47). Lastly, a long-term RCT (3 years) among postmenopausal women, with CIMT as the outcome, found no significant effects.[358] When RCT duration is taken into account, it is clear that intervention durations >6 months have been ineffective in improving cardiovascular outcomes.

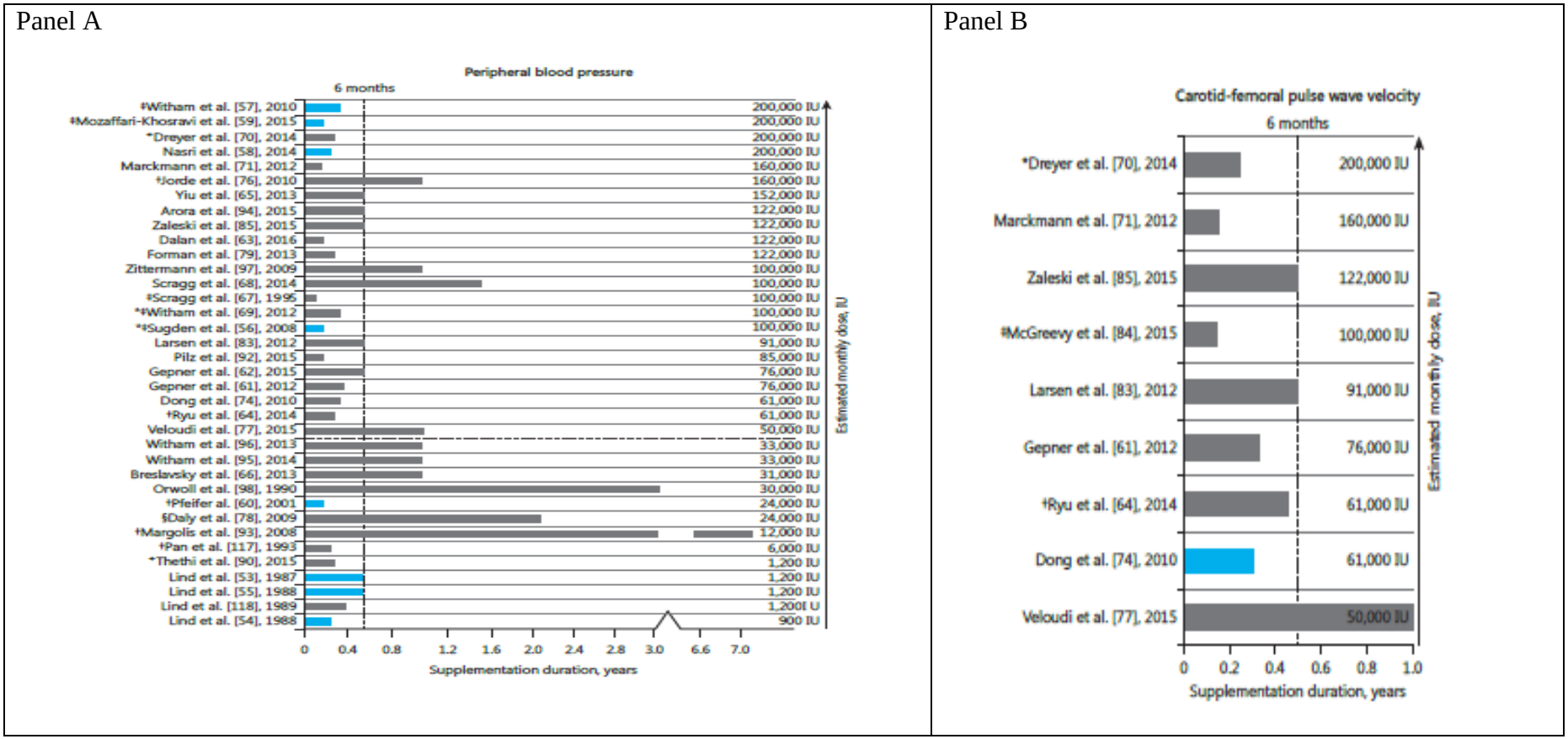

Figure 3. Randomised controlled trials (RCTs) on the effect of vitamin D supplementation according to trial duration and estimated monthly dose on brachial blood pressure (panel A) and carotid-femoral pulse wave velocity (panel B).

Randomised controlled trials (RCTs) on the effect of vitamin D supplementation according to trial duration and estimated monthly dose on brachial blood pressure (panel A) and carotid-femoral pulse wave velocity (panel B). Blue bars indicate effective RCTs (significant reduction in end points), and grey bars indicate RCTs that had no effect on end points. Black dotted horizontal line indicates adequate dose to raise serum vitamin D levels above deficiency levels. Black dotted vertical line indicates RCT duration of 6 months. Monthly dose was calculated using the highest dose if there was more than 1 vitamin D supplementation group. IU, international units. * Vitamin D_2 supplementation. † Vitamin D plus calcium. ‡ Single dose. § Vitamin D/calcium-fortified milk.

Baseline VitD Level

A series of rules regarding optimal design of RCTs examining nutrient effects has recently been introduced.[369] Rule 1 states that individuals with low baseline nutrient levels should be recruited. This is based on the pharmacological dose-response curve of nutrient intake; if baseline nutrient levels are deficient, then an increase in these levels is expected to produce a clinically meaningful and measurable effect on outcomes, whereas if baseline levels are within optimal ranges, then a further increase will produce no significant effects. Conversely, if baseline levels are high, then intervention could cause adverse effects due to toxicity. Overall, among the 45 RCTs included in this review, only 27% (*n* = 12) included patients with VitD deficiency (defined as <25 ng/ml; according to Mayo Medical Laboratories reference ranges).[370] The remaining 73% of studies placed no restrictions on baseline serum VitD levels or included a combination of deficient and non-deficient participants (i.e., <30,<40 or <60 ng/ml), which could confound the results. Among the RCTs that included VitD-deficient participants and investigated the effects on brachial BP (n = 8), 6 had no significant effects[342,346,347,350,357,365] and 2 (very short-term; ≤3 months) trials reported an improvement in brachial BP[165,337] (Table 1). None of the RCTs appropriately addressing rule 1 found a significant effect on cfPWV,[342,346,347,350,356,357] carotid-radial PWV[163] or brachial-ankle PWV.[342] With respect to AIx, among the trials that have recruited VitD-deficient participants, 2 short-term RCTs showed an improvement,[163,356] 2 RCTs with longer duration failed to find significant effects[342,350] and 1 RCT reported a paradoxical increase in AIx among individuals with T2DM.[341] FMD was improved in 1 RCT, 8 weeks after a single oral VitD dose,[337] but was not improved in 2 RCTs meeting rule 1.[360,361] In brief, VitD interventions that have recruited VitD-deficient participants generally failed to show significant treatment effects.

Adequate VitD Dose

Rules 2 and 3 of Heaney[369] require that the intervention dosage should be sufficient to change nutrient status (from deficient levels at baseline to sufficient levels at follow-up) and that the change in nutrient status must be reported. All (n = 12) but 1 RCT[357] that recruited VitD-deficient subjects were effective in increasing average VitD levels above deficiency, and all these RCTs reported the changes in serum VitD levels. In order to change baseline VitD levels from being deficient at baseline to being sufficient at follow-up, the dose of VitD should be adequate to raise serum VitD. Supplementation of 1,000--2,000 IU or lower, taken once or twice weekly (which translates to approximately 8,000--16,000 IU/month), has been shown to be insufficient for raising plasma 25-hydroxyvitamin D levels (the most objective biomarker for VitD nutritional adequacy)[371] higher than those of individuals who are not taking VitD supplements.[372] It is recommended that the VitD dose should be at least 50,000 IU/month in order to successfully raise baseline (deficient) serum VitD levels above 30 ng/mL (75 nmol/L; the lowest sufficient threshold).[373] Importantly, these recommendations are based on expectations for improving skeletal health outcomes and may not be relevant to cardiovascular related outcomes. Nonetheless, Figure 6. 3 shows clearly that irrespective of the dose, the majority of RCTs failed to improve brachial BP or cfPWV.

Rule 4 aims to ensure that the effect of a trial is a result of the change in the nutrient from the study intervention rather than a change in the diet. Although all 12 RCTs that met rules 1--3 reported changes in serum VitD levels, they did not state whether other steps were employed in order to monitor and adjust for issues related to adherence, such as variations in individual VitD absorption, changes in diet or other conditions that may have affected the change in serum VitD levels (i.e., physical activity, obesity or amount of exposure to sunlight).[192,193] Rule 5 considers confounding effects induced by changes in co-nutrient levels known to affect the outcomes (i.e., changes in serum calcium levels are monitored so that results are not biased by confounding effects). Among RCTs meeting rule 1--3, 7 studies monitored calcium and

phosphorous levels but did not adjust the final results for the changes in calcium levels. None of the RCTs meeting rules 1--3 adjusted the final outcomes for changes in these factors. Thus, it cannot be excluded that the results from RCTs that met rules 1--3 may have been confounded by significant uncontrolled factors influencing nutrient status.

High-Risk Populations

The interpretation of RCTs may also be complicated by the selection of individuals without a high-risk profile in terms of cardiovascular disease or VitD deficiency. Table 1 shows the effect of VitD interventions on brachial and cBP, cfPWV and AIx in various populations. It can be seen that VitD is ineffective in improving cardiovascular health, irrespective of the population risk profile. Further examination among those RCTs meeting rules 1--3 as per Heaney[369] shows that VitD supplementation is ineffective in VitD-deficient individuals with pre-hypertension, hypertension, T2DM or CKD. Our own work found that VitD supplementation was not beneficial among older individuals with VitD deficiency and osteoarthritis, a condition associated with increased cardiovascular a population enriched with vascular risk factors that should be most amenable to benefit from treatment.

Systematic Reviews and Meta-Analyses

Several systematic reviews concluded that VitD supplementation does not have a significant effect on systolic BP (included RCTs: $n = 46$;[352] $n = 8$;[374] $n = 10$;[375] $n = 16$),[376] whilst a small meta-analysis of 4 RCTs showed evidence of a statistically significant effect on systolic BP,[377] and others showed a statistically significant effect on diastolic BP (included RCTs: $n = 8$;[374] $n = 15$;[378] $n = 16$)[379]. At the same time, recent systematic reviews and meta-analyses concluded that VitD has no effect on markers of arterial stiffness (included RCTs: $n = 7$;[380] $n = 13$)[381] or markers of endothelial function (included RCTs: $n = 16$).[379] In alignment with this narrative review, almost all of the published systematic reviews highlight the significant methodological

heterogeneity in terms of dose and duration of treatment, which limits the interpretation of the findings and raises the need for a critical appraisal, over and beyond the reported results. Although some systematic reviews have used criteria to establish the quality of study design (i.e., allocation concealment, blinding, baseline comparability of groups, description of dropouts and intention-to-treat analysis), none of them have pooled the data according to the guidelines by Heaney.[369] Nevertheless, sensitivity analyses among the above analyses looking separately at either the effect of dose or duration of supplementation or baseline VitD status did not alter the null-effect findings.[352,379,381]

Conclusions

In summary, after consideration of the discrepancies in the study design of RCTs (i.e., sample size, duration of supplementation, selection of VitD-deficient subjects, VitD dose and population under investigation), VitD supplementation appears to be ineffective in improving brachial BP, large artery stiffness or central haemodynamics. This conclusion, though, cannot be generalised to other forms of contributions to endogenous VitD. The lack of evidence from large-scale RCTs with a priori primary measures of cardiovascular disease does not exclude the possibility of small but yet clinically meaningful VitD effects. Several large-scale RCTs in general populations (n >18,000; duration of 5 years) are underway and should provide a more definite answer to this question.[204] Despite a lack of large and long-term studies investigating atherosclerotic burden and endothelial function markers, evidence from RCTs also contradicts observational data that support a role of VitD in improving these markers. Thus, this review supports the notion that the inverse associations seen in observational studies between low VitD levels and cardiovascular end points are likely to be epiphenomena rather than true cause-and-effect relationships.

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